



US009468672B2

(12) **United States Patent**
Binder et al.

(10) **Patent No.:** US 9,468,672 B2
(45) **Date of Patent:** *Oct. 18, 2016

(54) **PROSTATE-ASSOCIATED ANTIGENS AND VACCINE-BASED IMMUNOTHERAPY REGIMENS**(71) Applicant: **Pfizer Inc.**, New York, NY (US)(72) Inventors: **Joseph John Binder**, San Diego, CA (US); **Helen Kim Cho**, San Diego, CA (US); **Michael Robert Dermeyer**, Carlsbad, CA (US); **Karin Ute Jooss**, San Diego, CA (US); **Brian Gregory Pierce**, Wayland, MA (US); **Joyce Tsai Tan**, Chapel Hill, NC (US); **Van To Tsai**, San Diego, CA (US)(73) Assignee: **Pfizer Inc.**, New York, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/657,302**(22) Filed: **Mar. 13, 2015**(65) **Prior Publication Data**

US 2015/0246106 A1 Sep. 3, 2015

Related U.S. Application Data

(62) Division of application No. 13/875,162, filed on May 1, 2013, now Pat. No. 9,066,898.

(60) Provisional application No. 61/642,844, filed on May 4, 2012.

(51) **Int. Cl.**

A61K 39/00	(2006.01)
C07H 21/04	(2006.01)
A61K 39/39	(2006.01)
A61K 39/395	(2006.01)
C12N 9/48	(2006.01)
A61K 31/404	(2006.01)
C07K 14/47	(2006.01)
C12N 9/64	(2006.01)

(52) **U.S. Cl.**

CPC	A61K 39/0011 (2013.01); A61K 31/404 (2013.01); A61K 39/39 (2013.01); A61K 39/395 (2013.01); C07K 14/4748 (2013.01); C12N 9/485 (2013.01); C12N 9/6445 (2013.01); C12Y 304/17021 (2013.01); A61K 2039/53 (2013.01); A61K 2039/55561 (2013.01)
-----------	--

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,538,866 A	7/1996	Israeli et al.
6,541,212 B2	4/2003	Reiter et al.

6,825,326 B2	11/2004	Reiter et al.
7,005,498 B1	2/2006	Steinaa
7,109,003 B2	9/2006	Hanson et al.
7,288,251 B2	10/2007	Bedian et al.
7,288,636 B2	10/2007	Mikolajczyk
8,435,516 B2	5/2013	Huang et al.
9,066,898 B2	6/2015	Binder et al.
2003/0086930 A1	5/2003	Mueller et al.
2005/0136055 A1	6/2005	Gladue et al.
2005/0226875 A1	10/2005	Gomez-Navarro et al.
2005/0272755 A1	12/2005	Denis et al.
2007/0059315 A1	3/2007	Jaffee et al.
2008/0193448 A1	8/2008	Baum et al.
2008/0279865 A1	11/2008	Gomez et al.
2009/0004213 A1	1/2009	Singh et al.
2009/0074787 A1	3/2009	Gomez et al.
2009/0117132 A1	5/2009	Readett et al.
2010/0305196 A1*	12/2010	Probst A61K 39/0011 514/44 R
2010/3005196	12/2010	Probst et al.
2012/0263677 A1	10/2012	Eagle et al.

FOREIGN PATENT DOCUMENTS

WO	WO2006048749	5/2006
WO	WO2007113648	10/2007
WO	WO20120042421	4/2012
WO	WO2012065164	5/2012
WO	WO2013006050	1/2013

OTHER PUBLICATIONS

Qin et al (Immunology Letter, 2005, 99:85-93, IDS).*
Waeckerle-Men et al (Cancer Immunol Immunother, 2006, 55:1524-1533).*

Schmittgen, T., et al., "Expression of Prostate Specific Membrane Antigen and Three Alternatively Spliced Variants of PSMA in Prostate Cancer Patients," Int. J. Cancer, 2003, vol. 107(2):323-9.
Schoenfeld, J. et al., "Active immunotherapy induces antibody responses that target tumor angiogenesis," Cancer Research, 2010, vol. 70:10150-10160.

Schroers, R., et al., "Identification of MHC class II-restricted T-cell epitopes in prostate-specific membrane antigen," Clinical Cancer Research, 2003, vol. 9(9):3260-71.

Sharma, R. et al., "4-1BB Ligand As an Effective Multifunctional Immunomodulator and Antigen Delivery Vehicle for the Development of Therapeutic Cancer Vaccines," Cancer Research, 2010 , vol. 70(10):3945-3954.

Shen, L. et al., "Cellular Protein Is the Source of Cross-Priming Antigen in Vivo," Proc Natl Acad Sci U S A 2004, vol. 101(9):3035-3040.

(Continued)

Primary Examiner — Laura B Goddard

(74) Attorney, Agent, or Firm — Austin W. Zhang

(57) **ABSTRACT**

The present disclosure provides (a) isolated immunogenic PAA polypeptides; (b) isolated nucleic acid molecules encoding immunogenic PAA polypeptides; (c) vaccine compositions comprising an immunogenic PAA polypeptide or an isolated nucleic acid molecule encoding an immunogenic PAA polypeptide; (d) methods relating to uses of the polypeptides, nucleic acid molecules, and compositions; and (e) vaccine-based immunotherapy regimens which involve co-administration of a vaccine in combination with an immune-suppressive-cell inhibitor and an immune-effector-cell enhancer.

(56)

References Cited**OTHER PUBLICATIONS**

- Sonpavde, G., et al., "Recent Advances in Immunotherapy for the Treatment of Prostate Cancer." *Expert Opinion on Biol. Ther.*, 2011, vol. 11(8):997-1009.
- Stura E., et al., "Crystal Structure of Human Prostate-Specific Antigen in a Sandwich Antibody Complex," *Journal of Molecular Biology*, 2011, vol. 414:530-544.
- Terasawa, H. et al., "Identification and Characterization of a Human Agonist Cytotoxic T-Lymphocyte Epitope of Human Prostate-specific Antigen," *Clinical Cancer Research*, 2002, vol. 8:41-53.
- Thomas-Kaskel, A., et al., "Vaccination of Advanced Prostate Cancer Patients With PSCA and PSA Peptide-Loaded Dendritic Cells Induces DTH Responses That Correlate With Superior Overall Survival," *International Journal against Cancer*, 2006, vol. 119, 2428-2434.
- van den Eertwegh, A., et al., "Combined Immunotherapy With Granulocyte-Macrophage Colony-Stimulating Factor-Transduced Allogeneic Prostate Cancer Cells and Ipilimumab in Patients With Metastatic Castration-Resistant Prostate Cancer: A Phase 1 Dose-Escalation Trial," *Lancet Oncology*, 2012, vol. 13:509-17.
- Villoutreix, B., et al., "A Structural Model for the Prostate Disease Marker, Human Prostate-Specific Antigen," *Protein Science*, 1994, vol. 3(11):2033-2044.
- Zhang, J., et al., "A Small Composite Probasin Promoter Confers High Levels of Prostate-Specific Gene Expression Through Regulation by Androgens and Glucocorticoids In Vitro and In Vivo" *Endocrinology*, 2000, vol. 141 (12):4698-4710.
- Houot, R., et al., "T-cell modulation combined with intratumoral CpG cures lymphoma in a mouse model without the need for chemotherapy," *Blood*, 2009, 3546-3552, vol. 113.
- Olson, W., et al., "Clinical Trials of Cancer Therapies Targeting Prostate-Specific Membrane Antigen," *Reviews on Recent Clinical Trials*, 2007, 182-190, vol. 2.
- Ying, W., et al., "Dendritic cell-based multi-epitope immunotherapy of hormone-refractory prostate carcinoma", *Cancer Immunology Immunotherapy*, 2006, 1524-1533, vol. 55.
- Suttmuller, R., et al., "Synergism of Cytotoxic T Lymphocyte-associated Antigen 4 Blockade and Depletion of CD25 Regulatory T Cells in Antitumor Therapy Reveals Alternative Pathways for Suppression of Autoreactive Cytotoxic T Lymphocyte Responses", *J. Exp. Med.*, 2001, 823-832, vol. 194, No. 6.
- Ahlers, J., et al., "A push-pull approach to maximize vaccine efficacy: Abrogating suppression with an IL-13 inhibitor while augmenting help with granulocyte/macrophage colony-stimulating factor and CD40L", *PNAS*, 2002, 13020-13025, vol. 99, No. 20.
- Berzofsky, J., et al., "Progress on new vaccine strategies for the immunotherapy and prevention of cancer", *The Journal of Clinical Investigation*, 2004, 1515-1525, vol. 113, No. 11.
- Mangsbo, S., et al., "Enhanced Tumor Eradication by Combining CTLA-4 or PD-1 Blockade with CpG Therapy," *Journal of Immunotherapy*, 2010, 225-235, vol. 33.
- Database UniProt [Online]. "Glutamate carboxypeptidase 2; EC=3.4.17.21; Cell growth-inhibiting gene 27 protein; Folate hydrolase 1; Folylpoly-gama-glutamate carboxypeptidase", retrieved from EBI accession No. UNIPROT:Q04609 Database accession No. Q04609 sequence, Jun. 1, 1994.
- Ahmad, S. et al., "Prostate Stem Cell Antigen DNA Vaccination Breaks Tolerance to Self-Antigen and Inhibits Prostate Cancer Growth," *American Society of Gene Therapy*, 2009; vol. 17(6):1101-8.
- Arlen, P. et al., "Clinical Safety of a Viral Vector Based Prostate Cancer Vaccine Strategy." *The Journal of Urology*, 2007, vol. 178:1515-1520.
- Balk, S. et al., "Biology of Prostate-Specific Antigen," *Journal of Clinical Oncology*, 2003, vol. 21(2):383-391.
- Bar'inka, C. et al., "Amino Acids At the N- and C-Termini of Human Glutamate Carboxypeptidase II Are Required for Enzymatic Activity and Proper Folding," *Eur. J. Biochem*, 2004, vol. 271:2782-2790.
- Barinka, C. et al. "Identification of the N-glycosylation sites on glutamate carboxypeptidase II necessary for proteolytic activity." *Protein Science*, 2004, vol. 13(6): 1627-1635.
- Bose, A. et al., "Combined Vaccine + Axitinib Therapy Yields Superior Antitumor Efficacy in a Murine Melanoma Model," *Melanoma Research*, 2012, vol. 22:236-243.
- Cha, E., et al., "Therapeutic Vaccines for Prostate Cancer." *Current Opinion in Molecular Therapeutics*, 2010, vol. 12 (1): 77-85.
- Chakraborty, M. et al., "The Combined Activation of Positive Costimulatory Signals With Modulation of a Negative Costimulatory Signal for the Enhancement of Vaccine-Mediated T-Cell Responses," *Cancer Immunol Immunother*, 2007, vol. 56:1471-1484.
- Collins, D., et al., "Trastuzumab Induces Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) in HER-2-Non-Amplified Breast Cancer Cell Lines" *Annals of Oncology*, 2011, vol. 23:1788-1795.
- Dall'Ozzo, S., et al., "Rituximab-Dependent Cytotoxicity by Natural Killer Cells: Influence of FCGR3A Polymorphism on the Concentration-Effect Relationship." *Cancer Research*, 2004, vol. 64(13): 4664-4669.
- Dannull J. et al., "Prostate Stem Cell Antigen is a Promising Candidate for Immunotherapy of Advanced Prostate Cancer," *Cancer Research*, 2000, vol. 60:5522-28.
- Davis, M., et al., "Crystal Structure of Prostate-Specific Membrane Antigen, a Tumor Marker and Peptidase," *Proc Natl Acad Sci*, 2005, vol. 102(17):5981-6.
- Drake, C. et al., "Update: immunological strategies for prostate cancer," *Curr Urol Rep*, 2010, vol. 11(3):202-7.
- Durso, R., et al., "A Novel Alphavirus Vaccine Encoding Prostate-Specific Membrane Antigen Elicits Potent Cellular and Humoral Immune Responses." *Clinical Cancer Research*, 2007, vol. 13(13):3999-4008.
- Elsasser-Beile, U., et al., "Targeted Therapies for Prostate Cancer Against the Prostate Specific Membrane Antigen," *Current Drug Targets*, 2009, vol. 10(2):118-25.
- Farsaci, B. et al., "Consequence of Dose Scheduling of Sunitinib on Host Immune Response Elements and Vaccine Combination Therapy" *International Journal of Cancer*, 2011, vol. 0:1-12.
- Ferraro, B., et al., "Co-delivery of PSA and PSMA DNA Vaccines With Electroporation Induces Potent Immune Responses," *Human Vaccines*, 2011, vol. 7:120-127.
- Garcia-Hernandez , M., et al., "Prostate Stem Cell Antigen Vaccination Induces a Long-Term Protective Immune Response Against Prostate Cancer in the Absence of Autoimmunity." *Cancer Research*, 2008, vol. 68(3): 861-869.
- Goodman, O., et al. "Interaction of prostate specific membrane antigen with clathrin and the adaptor protein complex-2," *International Journal of Oncology* , 2007, vol. 31(5): 1199-1203.
- Gregor, P., et al., "Induction of autoantibodies to syngeneic prostate-specific membrane antigen by xenogeneic vaccination," *International Journal of Cancer*, 2005, vol. 116(3): 415-21.
- Gu, Z., et al., "Prostate Stem Cell Antigen (PSCA) Expression Increases With High Gleason Score, Advanced Stage and Bone Metastasis in Prostate Cancer." *Oncogene*, 2000, vol. 19(10): 1288-1296.
- Harada, M., et al., "Prostate-Specific Antigen-Derived Epitopes Capable of Inducing Cellular and Humoral Responses in HLA-A24+ Prostate Cancer Patients," *The Prostate*, 2003, vol. 57:152-159.
- Hirao, L. et al., "Immune Modulation through 4-1BB Enhances SIV Vaccine Protection in Non-Human Primates against SIVmac251 Challenge," *PLoS One*, 2011, vol. 6(9): 1-11.
- Hodge, J., et al., "Multiple Costimulatory Modalities Enhance CTL Activity." *The Journal of Immunology*, 2005, vol. 174:5994-6004.
- Horig , H. et al., "Prostate-Specific Antigen Vaccines for Prostate Cancer," *Expert Opinion*, 2002, vol. 2(4):395-408.
- Houghton, C., et al., "Immunological validation of the EpitOptimizer program for streamlined design of heteroclitic epitopes." *Vaccine*, 2007, vol. 25(29): 5330-5342.
- Karan, D., et al., "Dual Antigen Target-Based Immunotherapy for Prostate Cancer Eliminates the Growth of Established Tumors in Mice." *Immunotherapy*, 2011, vol. 3(6): 735-746.

(56)

References Cited**OTHER PUBLICATIONS**

- Keler, T. et al., "Activity and Safety of CTLA-4 Blockade Combined with Vaccines in Cynomolgus Macaques," *The Journal of Immunology*, 2003, vol. 171:6251-6259.
- Kiesling, A., et al., "Advances in specific immunotherapy for prostate cancer," *European Urology*, 2008, vol. 53 (4):694-708.
- Kim, S., et al., "Vaccination With Recombinant Adenoviruses and Dendritic Cells Expressing Prostate-Specific Antigens Is Effective in Eliciting CTL and Suppresses Tumor Growth in the Experimental Prostate Cancer." *The Prostate*, 2009, vol. 69(9): 938-948.
- Kobayashi, K., et al., "Identification of a Prostate-Specific Membrane Antigen-Derived Peptide Capable of Eliciting Both Cellular and Humoral Immune Responses in HLA-A24+ Prostate Cancer Patients," *Cancer Sci*, 2003, vol. 94(7): 622-7.
- Kumar, A., et al. "Expression of Pro Form of Prostate-Specific Antigen by Mammalian Cells and Its Conversion to Mature, Active Form by Human Kallikrein 2," *Cancer Research*, 1997, vol. 57(15): 3111-3114.
- Li, Betty et al., "Established B16 Tumors Are Rejected Following Treatment With GM-CSF-Secreting Tumor Cell Immunotherapy in Combination With Anti-4-1BB Mab," *Clinical Immunology*, 2007, vol. 125:76-87.
- Li, Y. et al "Cytotoxicity of Human Prostate Cancer Cell Lines In Vitro and Induction of Apoptosis Using 213Bi-Herceptin Alpha-Conjugate." *Cancer Letters*, 2004, vol. 205(2): 161-171.
- Li, Y., et al "Promising Tumor-Associated Antigens for Future Prostate Cancer Therapy." *Medicinal Research Reviews*, 2010, vol. 30(1):67-101.
- Lu, J., et al. "Recognition of Prostate Tumor Cells by Cytotoxic T Lymphocytes Specific for Prostate-Specific Membrane Antigen," *Cancer Research*, 2002, vol. 62(20):5807-12.
- Lubaroff, D., et al., "Phase I Clinical Trial of an Adenovirus/Prostate-Specific Antigen Vaccine for Prostate Cancer: Safety and Immunologic Results." *Clinical Cancer Research*, 2009, vol. 15(23): 7375-7380.
- Lundwall, A., et al., "Molecular Cloning of Human Prostate Specific Antigen cDNA," *FEBS Letters*, 1987, vol. 214(2): 317-322.
- Madan, R.. et al., "Ipilimumab and a Poxviral Vaccine Targeting Prostate-Specific Antigen in Metastatic Castration-Resistant Prostate Cancer: A Phase I Dose-Escalation Trial," *Lancet Oncol*, 2012; 13: 501-08.
- Matsueda, S., et al., "Identification of peptide vaccine candidates for prostate cancer patients with HLA-A3 supertype alleles," *Clinical Cancer Research*, 2005, vol. 11:6933-43.
- McCormack, R., et al., "Molecular Forms of Prostate-Specific Antigen and the Human Kallikrein Gene Family: A New Era." *Urology*, 1995, vol. 45(5):729-744.
- Mincheff, M., et al., "Immune Responses Against PSMA After Gene-Based Vaccination for Immunotherapy-A: Results From Immunizations in Animals," *Cancer Gene Therapy*, 2006, 13(4):436-44.
- Qin, H., et al., "Specific Antitumor Immune Response Induced by a Novel DNA Vaccine Composed of Multiple CTL and T Helper Cell Epitopes of Prostate Cancer Associated Antigens," *Immunology Letters*, 2005, vol. 99(1):85-93.
- Raff A., et al., "Prostate Stem Cell Antigen: A Prospective Therapeutic and Diagnostic Target," *Cancer Letters*, 2009, vol. 277:126-132.
- Rajasekaran, S., et al., "A novel cytoplasmic tail MXSSL motif mediates the internalization of prostate-specific membrane antigen," *Molecular Biology of the Cell*, 2003, vol. 14(12):4835-4845.
- Reiter, R., et al., "Prostate Stem Cell Antigen: A Cell Surface Marker Overexpressed in Prostate Cancer." *Proc Natl Acad Sci U S A* , 1994, vol. 91(4):1735-1740.
- Rock, K., et al., "Cross-Presentation: Underlying Mechanisms and Role in Immune Surveillance." *Immunology Reviews*, 2005, vol. 207: 166-183.
- Rountree, R., et al., "Exosome targeting of tumor antigens expressed by cancer vaccines can improve antigen immunogenicity and therapeutic efficacy." *Cancer Research*, 2011, vol. 71:5235-5244.
- Sacha, P., et al., "Expression of glutamate carboxypeptidase II in human brain," *Neuroscience*, 2007, vol. 144 (4):1361-72.
- Sakamoto, H. et al., "Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer," *Nature Genetics*, 2008, vol.;40(6):730-40.
- U.S. Appl. No. 14/527,226, filed Oct. 29, 2014.

* cited by examiner

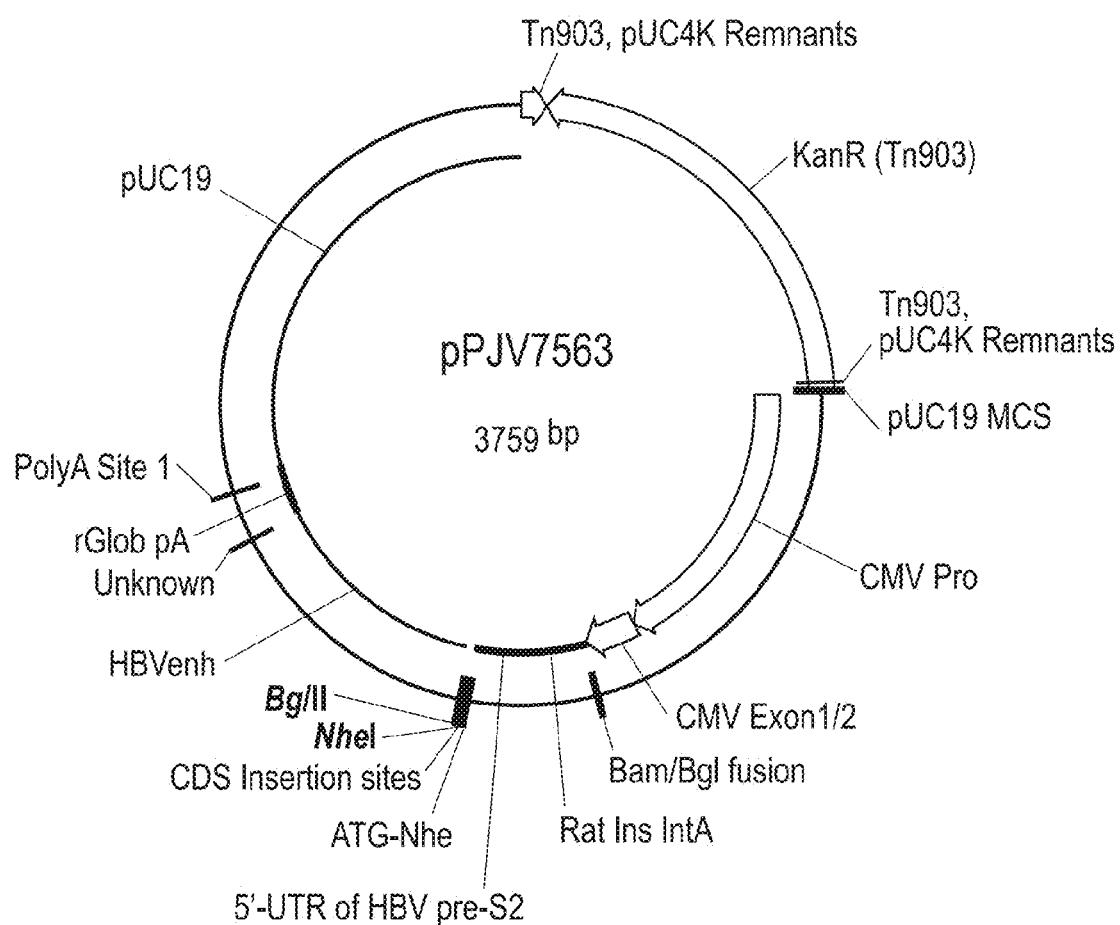
FIG. 1

FIG. 2

10	20	
-----+-----		
Q T L N F D L L K L A G D V E S N P G * P	FMDV 2A	
- - E G R G S L L T C G D V E E N P G * P	TAV 2A	
H Y A G Y F A D L L I H D I E T N P G * P	EMCV 2A	
Q C T N Y A L L K L A G D V E S N P G * P	ERAV 2A	
- A T N F S L L K Q A G D V E E N P G * P	PTV 2A	

FIG. 3

5' UAACGUUACUGGCCGAAGCCGUUGGAAUAAGGCCGGUGUGCUUUGCUAU
AUGUUAUUUUCCACCAUAUUGCUCUUUGCAAUGUGAGGGCCCGAACCU
GGCCCUGUCUUCUUGACGAGCAUUCUAGGGUCUUCCCCUCUCGCCAAAGG
AAUGCAAGGUCUGUUGAAUGUCUGAAGGAAGCAGUUCUCUGGAAGCUUU
GAAGACAAACAACGUCUGUAGCGACCCUUUGCAGGCAGCGAACCCCCCACCUG
GCGACAGGUGCCUCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUGCAAAGG
CGGCACAACCCCAGUGCCACGUUGUGAGUUGAUAGUUGUGGAAAGAGUAAA
GGCUCUCUCAAGCGUAUUCACAAGGGCUGAAGGAUGCCCAGAAGGUACCCC
AUUGUAUGGGAUCUGAUCUGGGGCCUCGGUGCAC AUGCUUUACAUGUGUUUAG
UCGAGGUUAAAAACGUCUAGGCCCGAACCACGGGACGUGGUUUUCCUU
GAAAAACACGAUGAUAAU*AUGGCCACAACCAUG3'

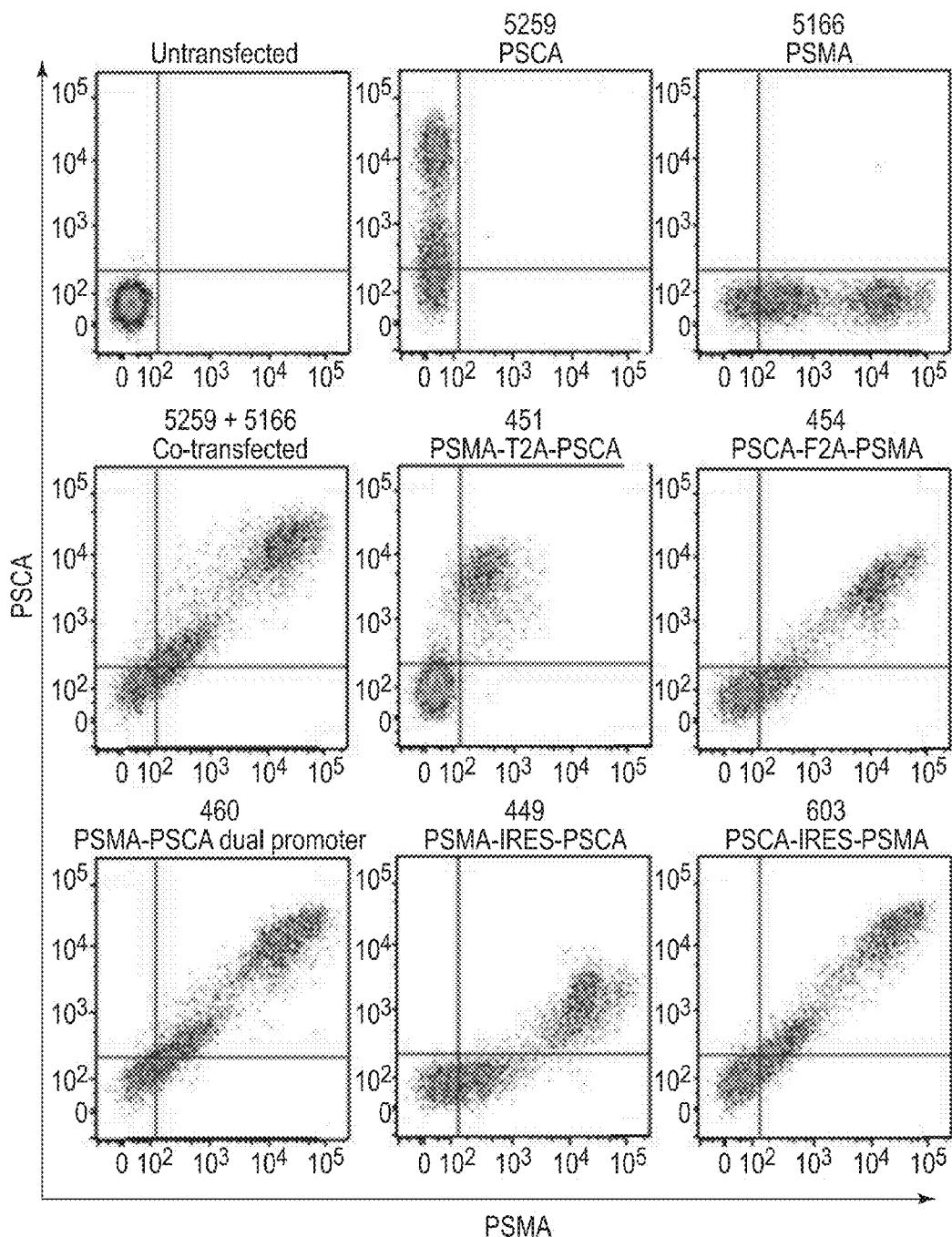
FIG. 4

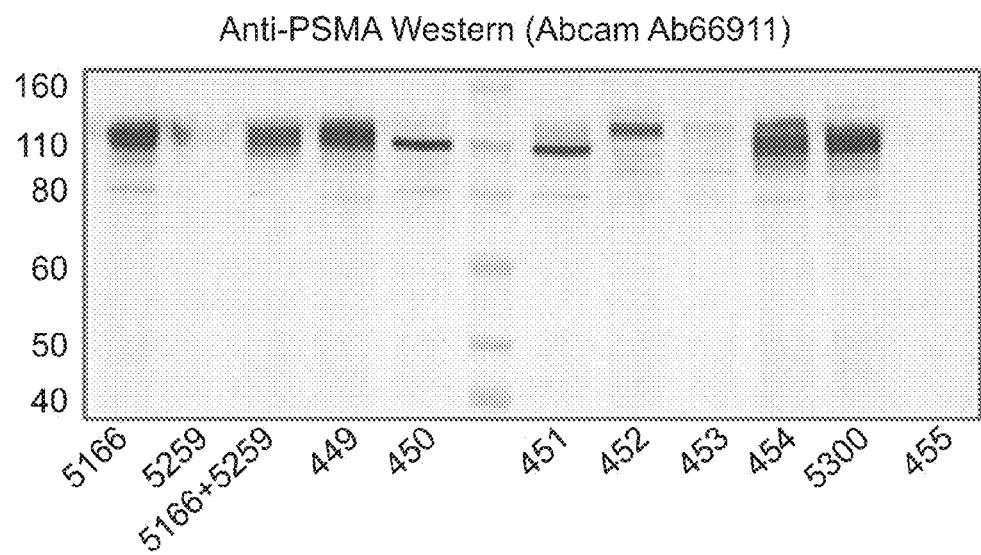
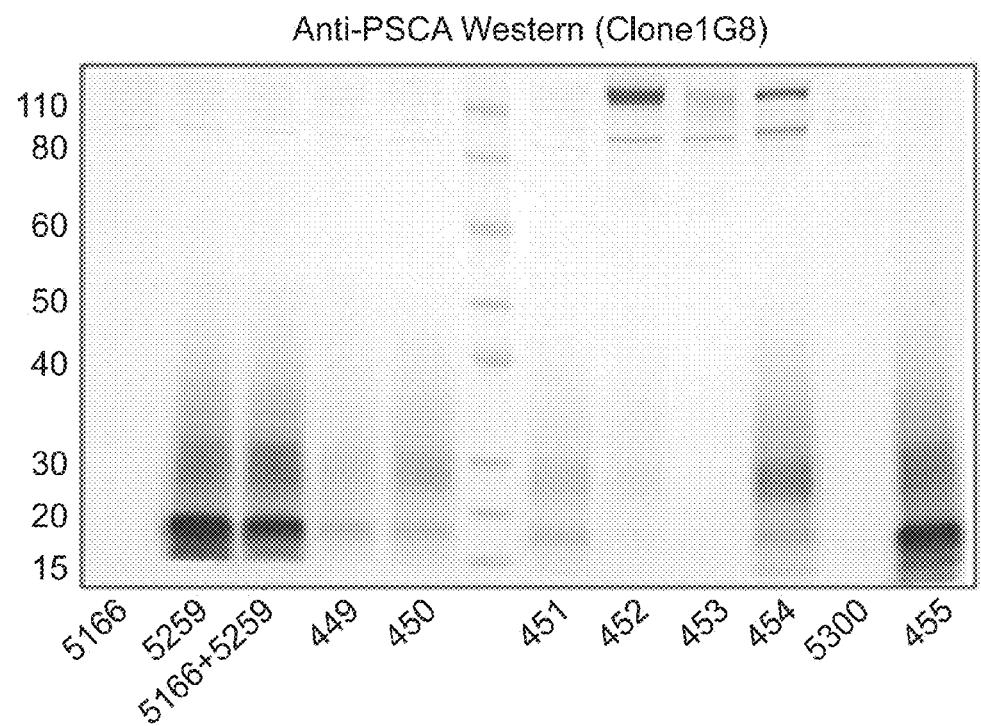
FIG. 5A**FIG. 5B**

FIG. 6

Anti-PSA Western (Ab46976)

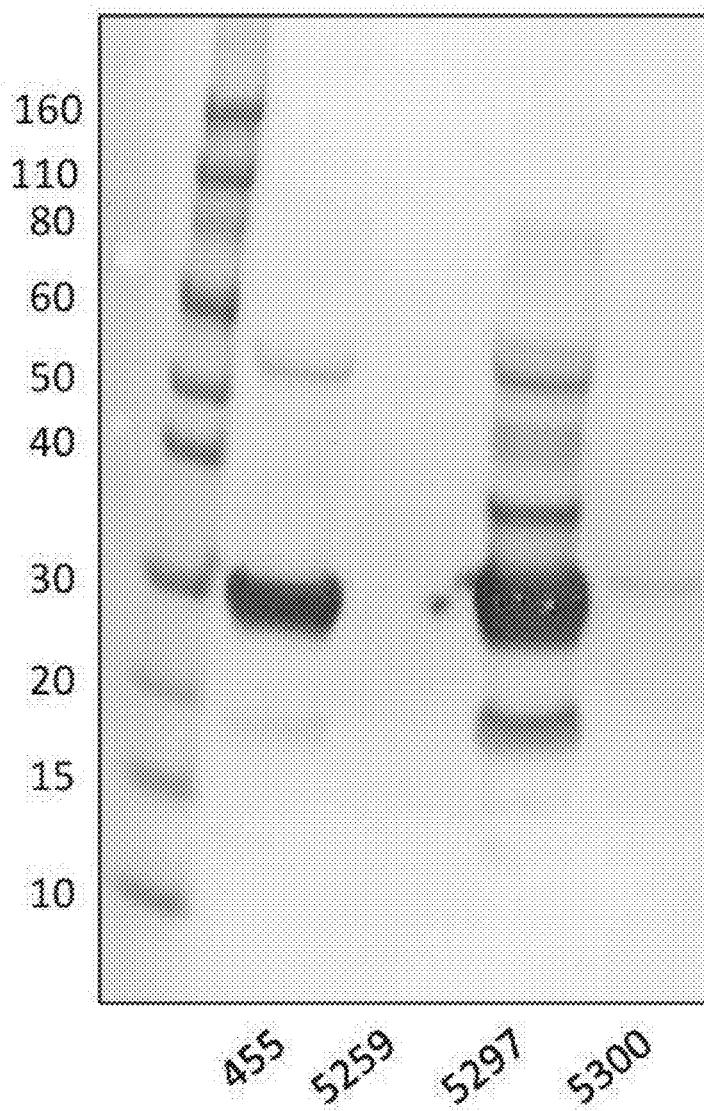


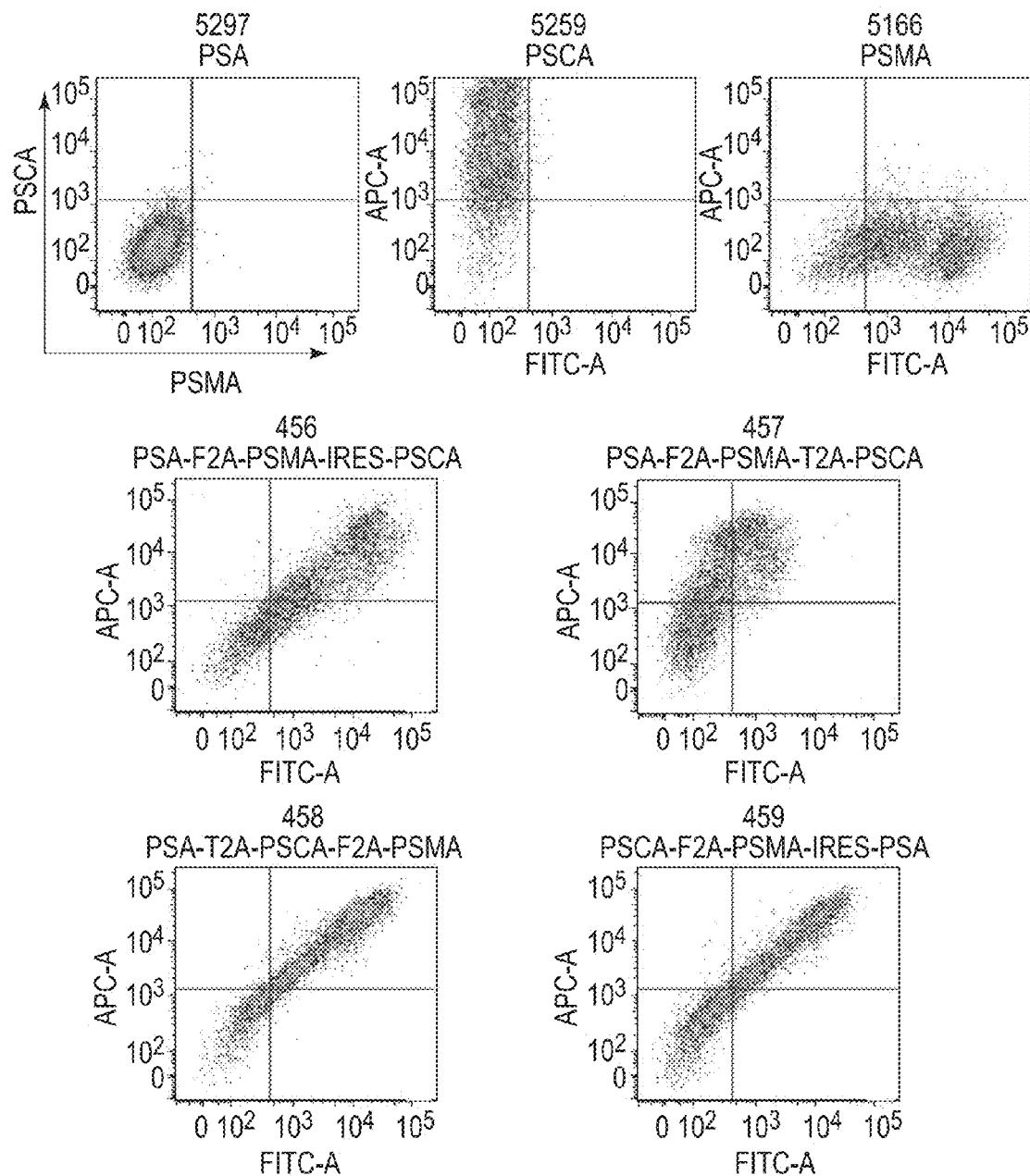
FIG. 7A

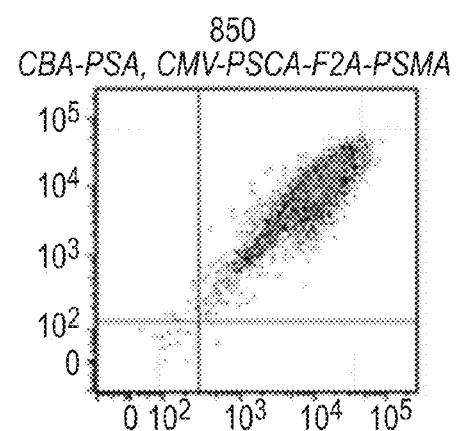
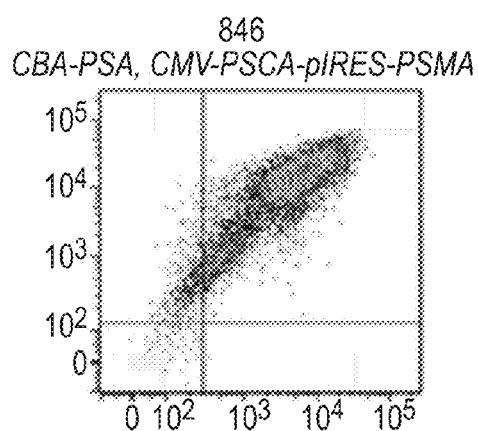
FIG. 7B

FIG. 8A

Anti-PSA Western (Ab46976)

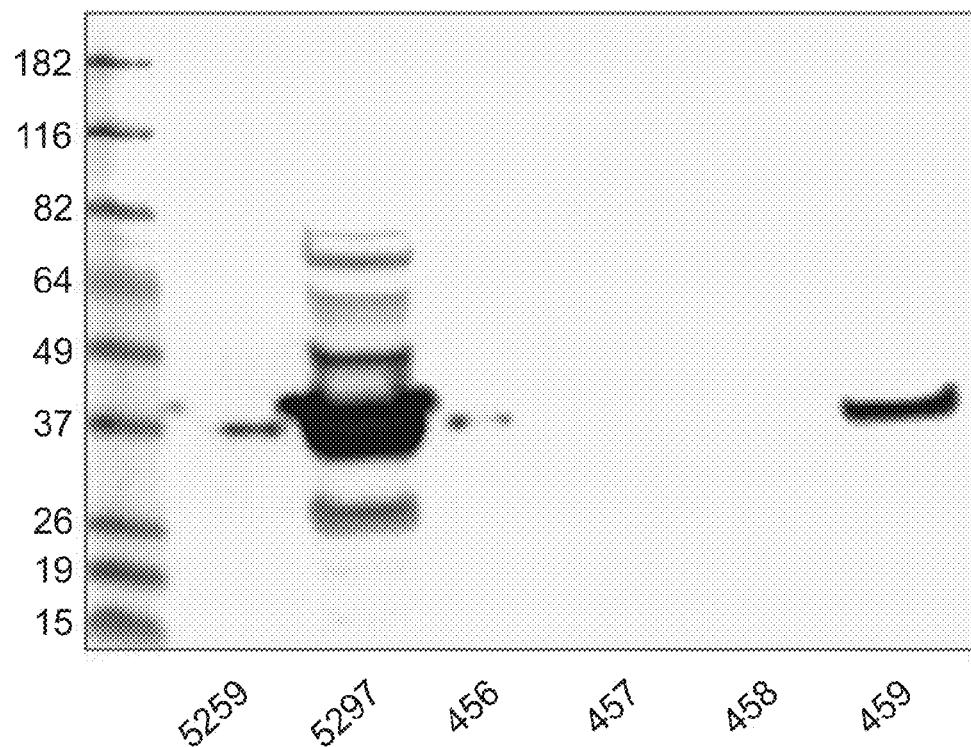
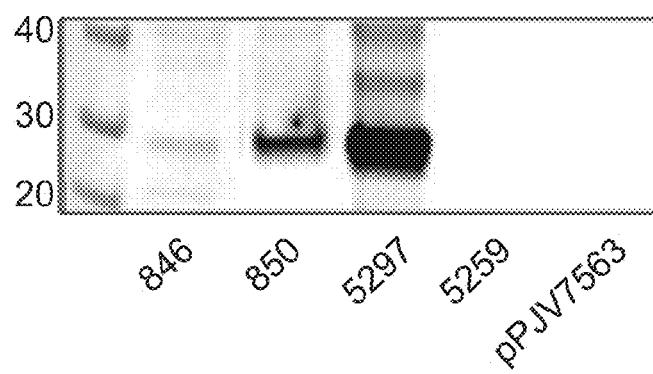
**FIG. 8B**

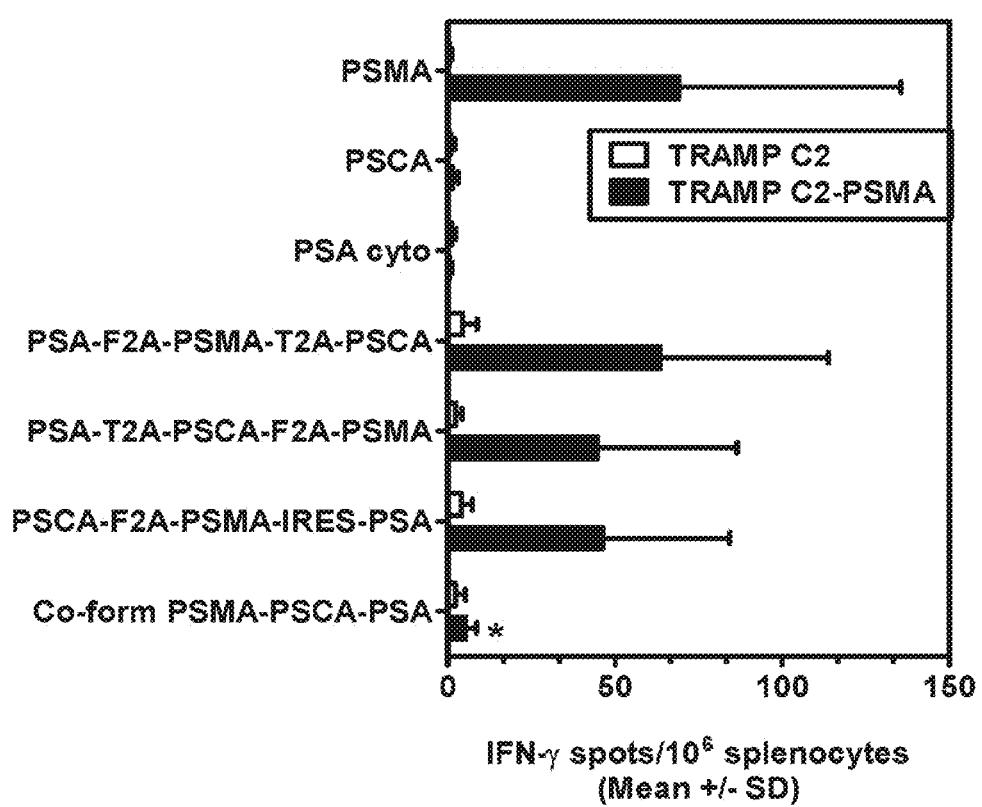
FIG. 9A

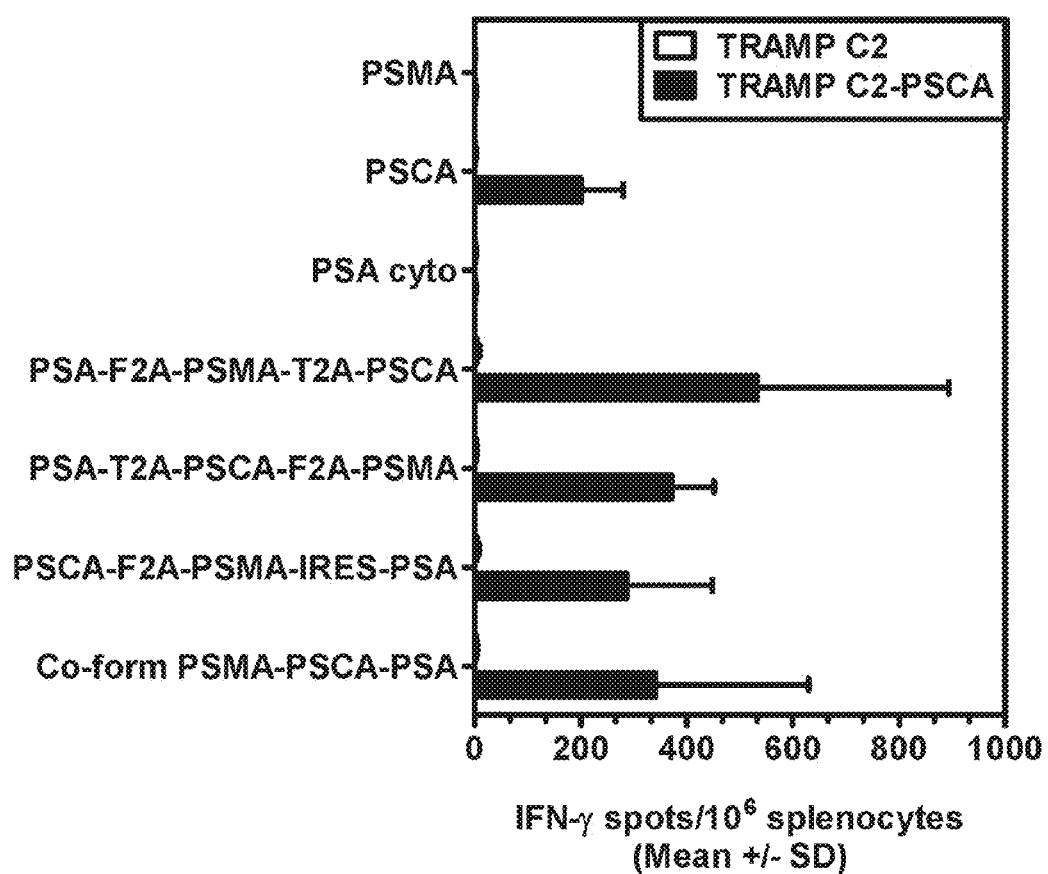
FIG. 9B

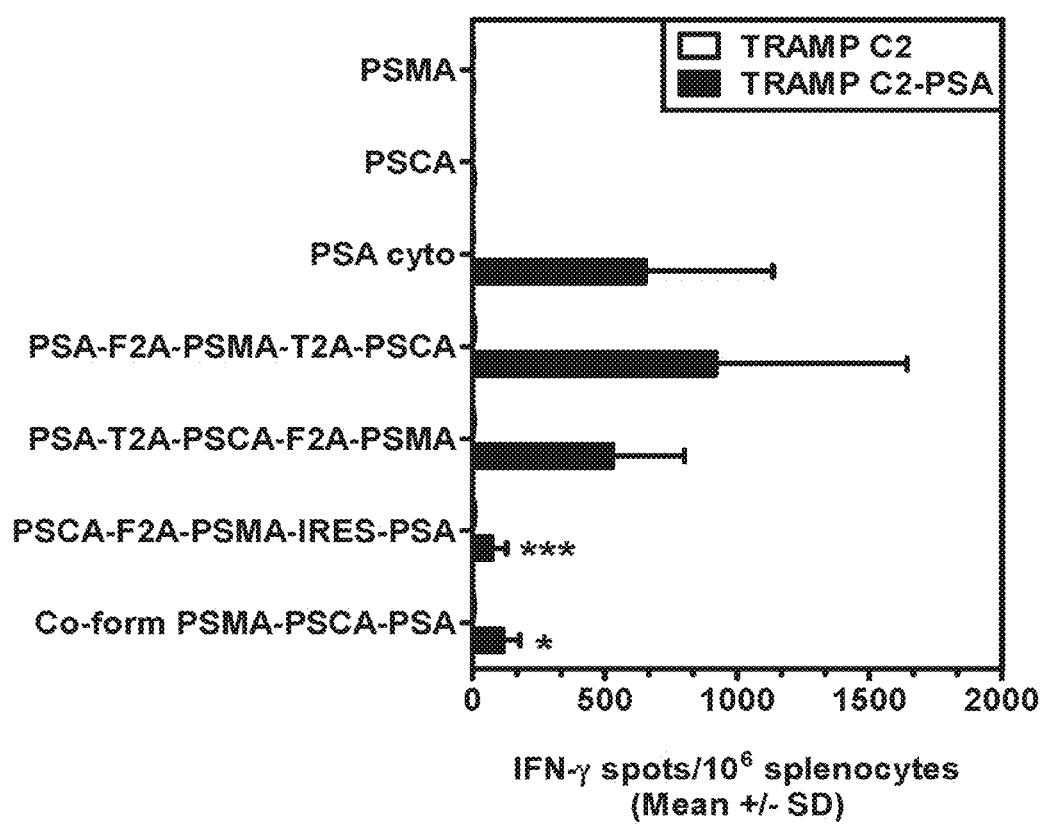
FIG. 9C

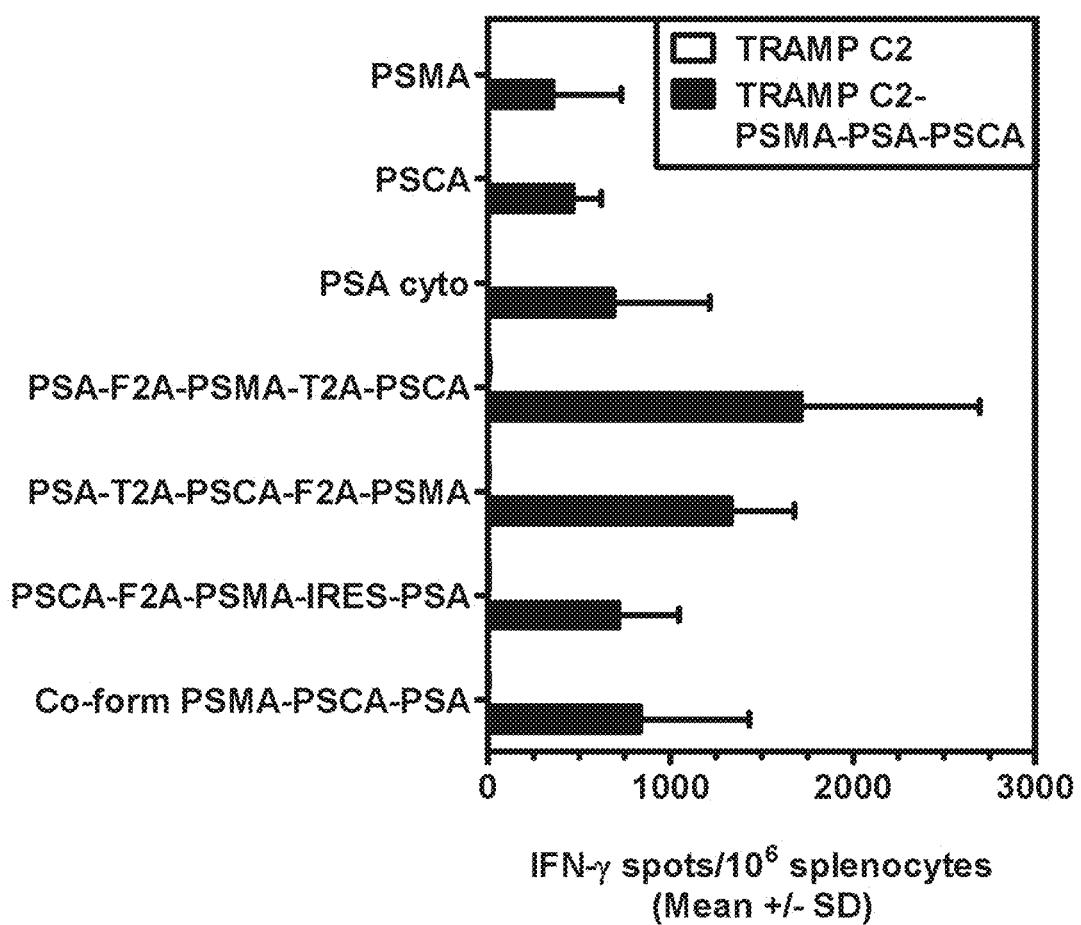
FIG. 9D

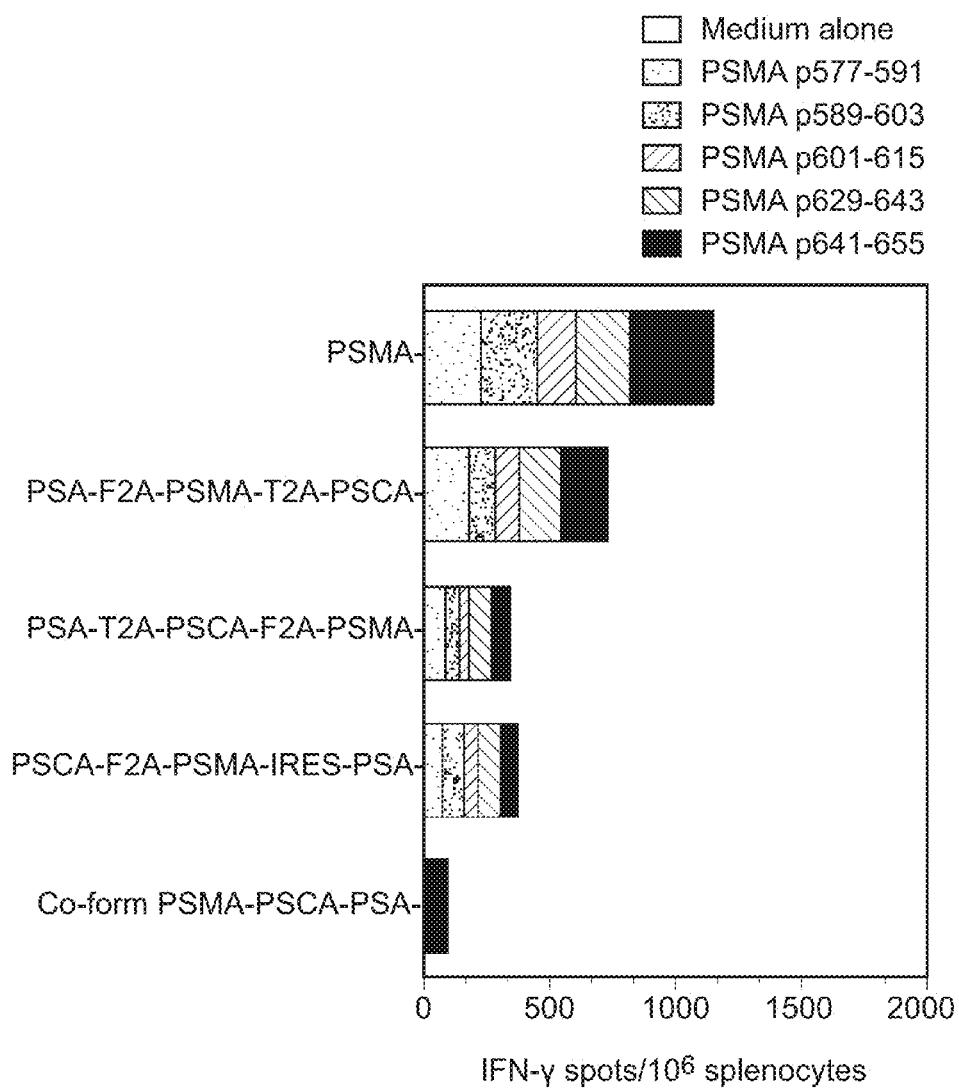
FIG. 10A

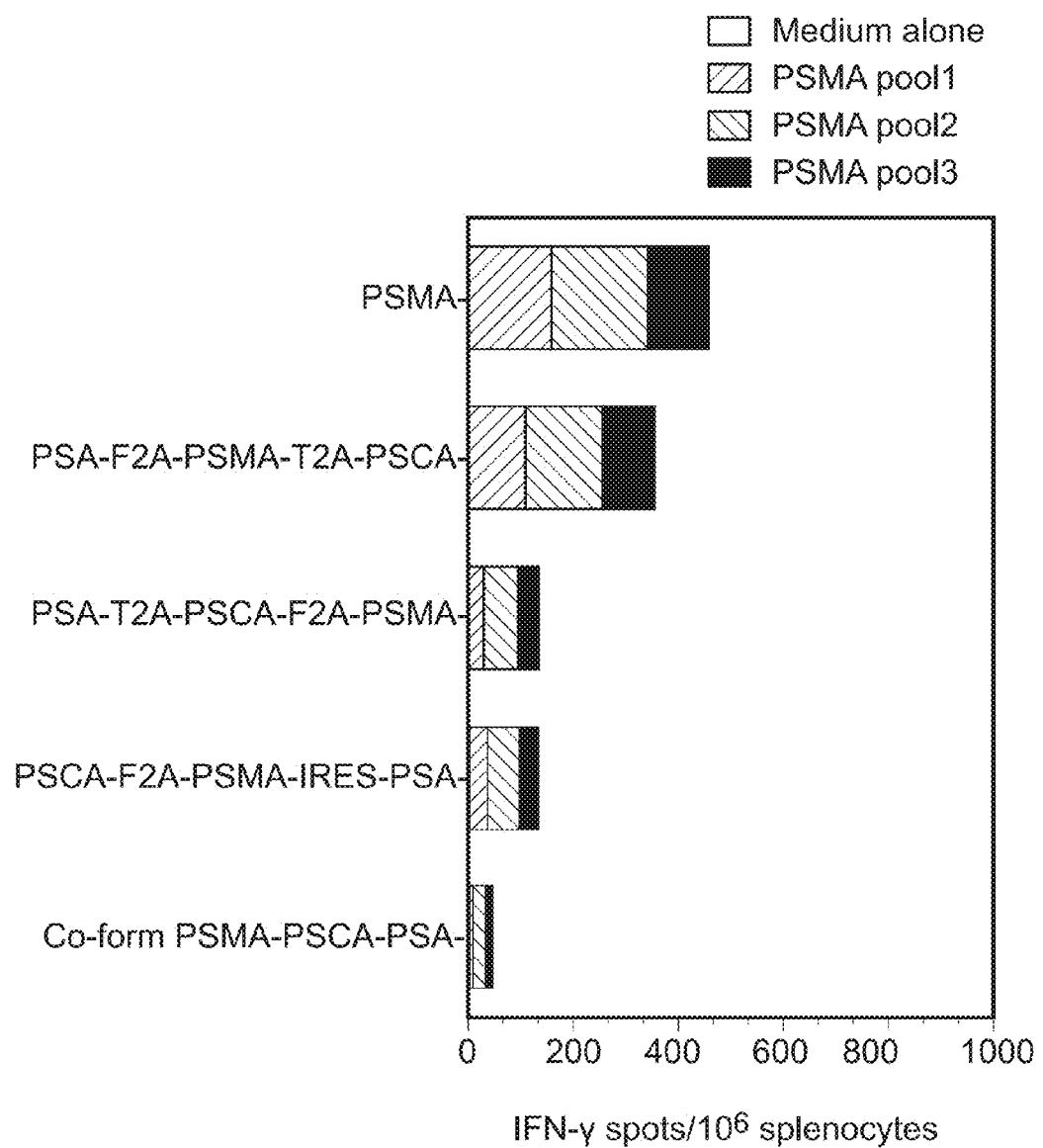
FIG. 10B

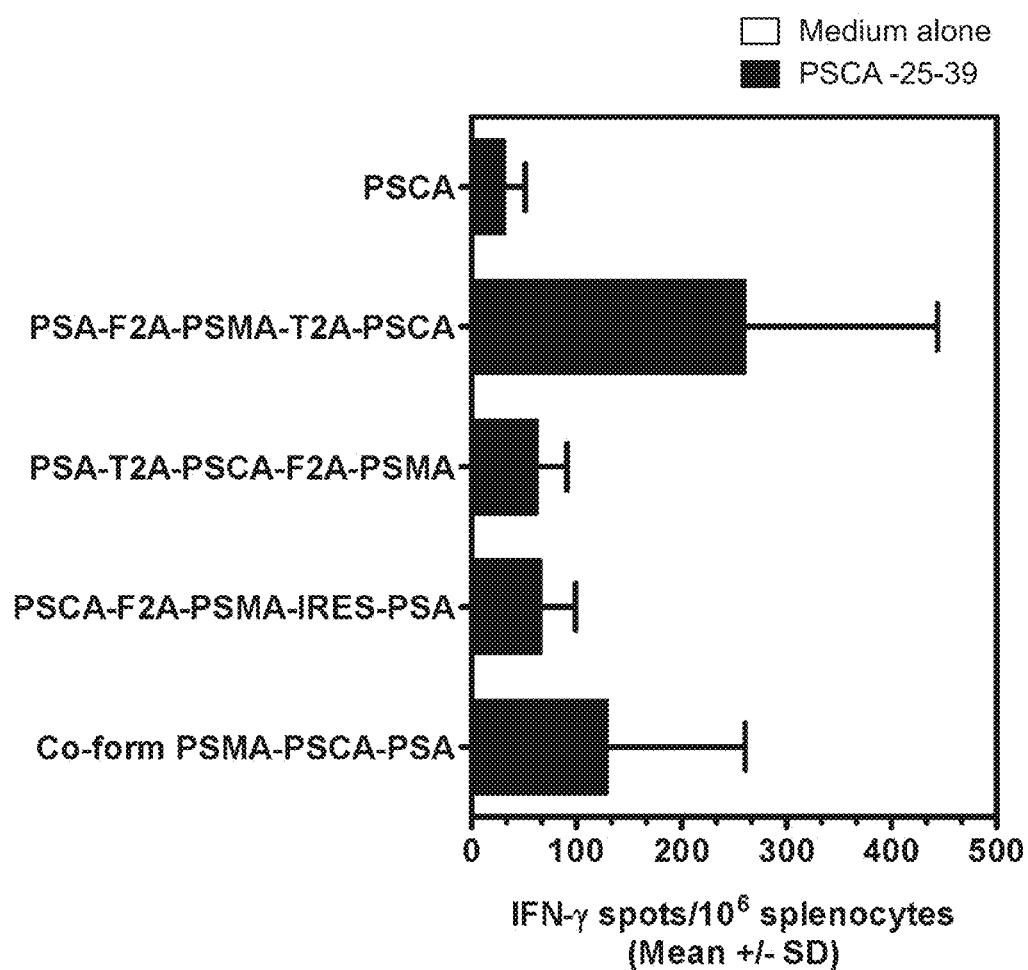
FIG. 10C

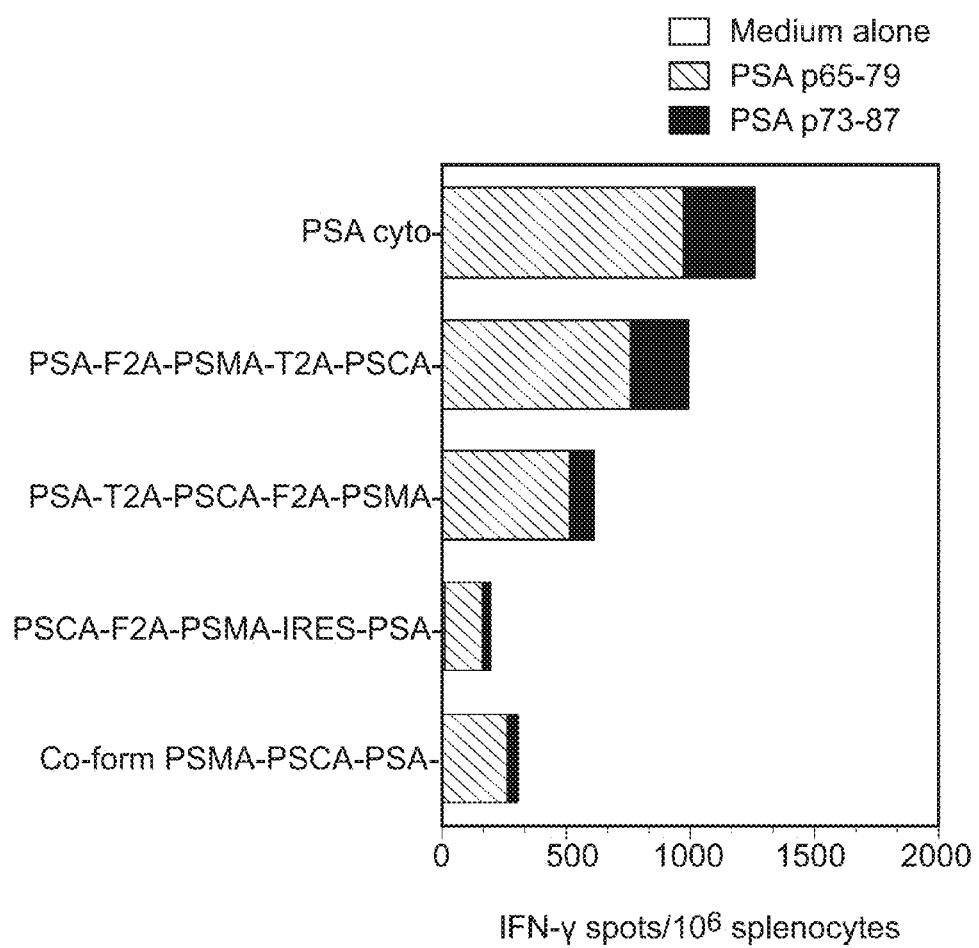
FIG. 10D

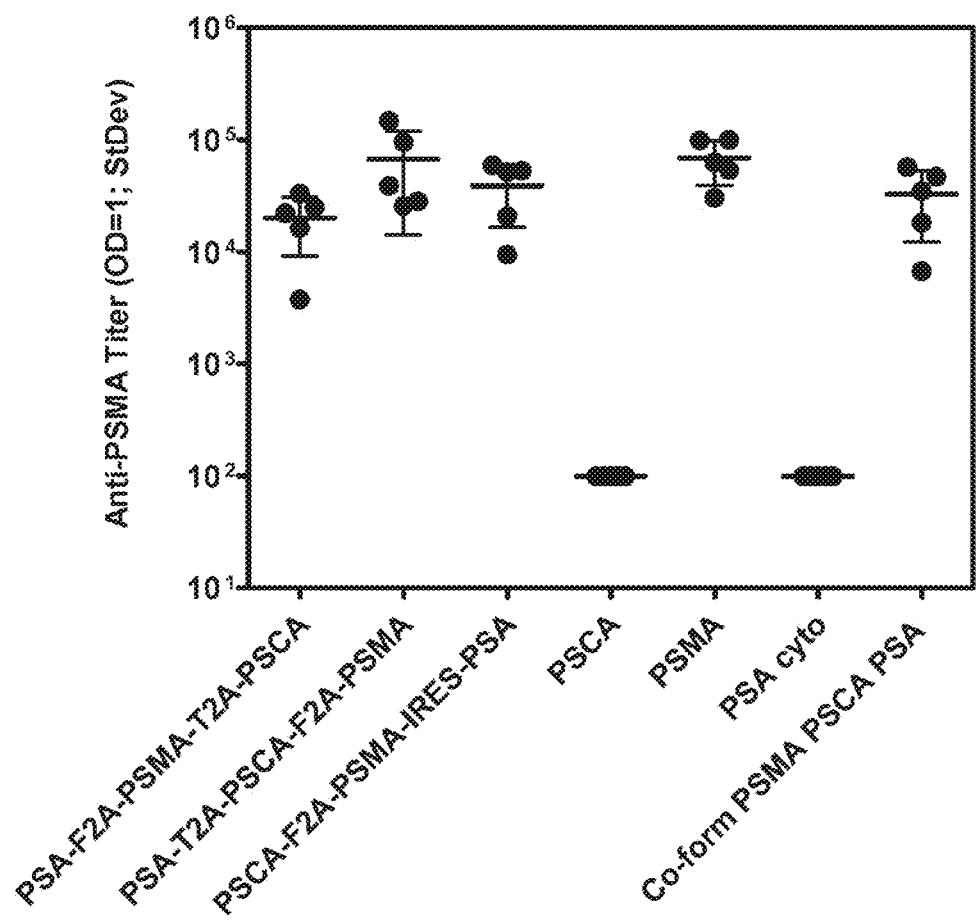
FIG. 11

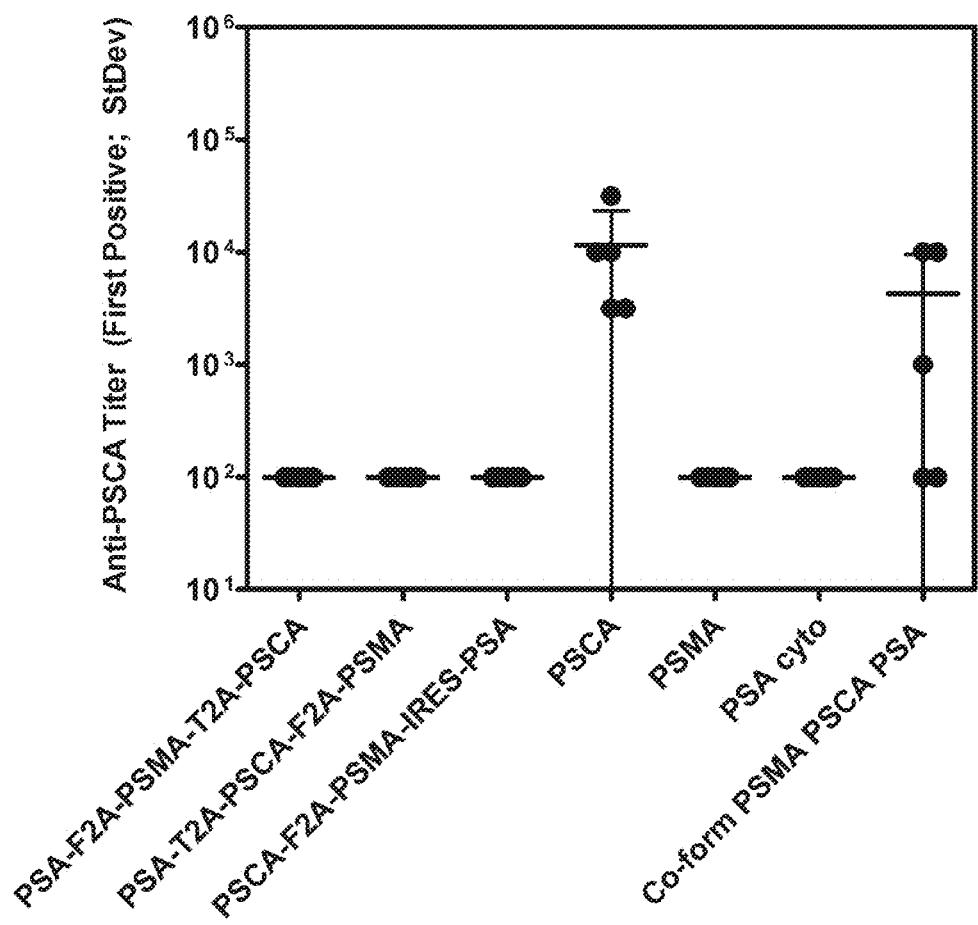
FIG. 12

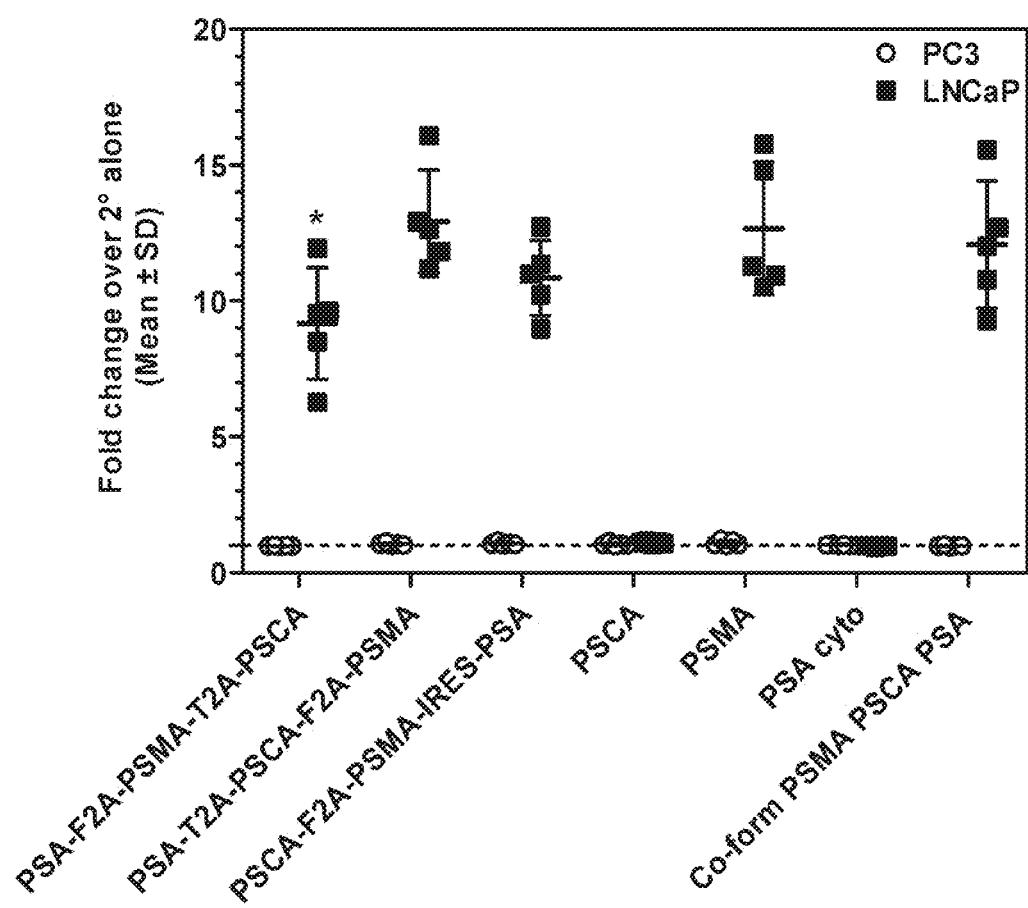
FIG. 13

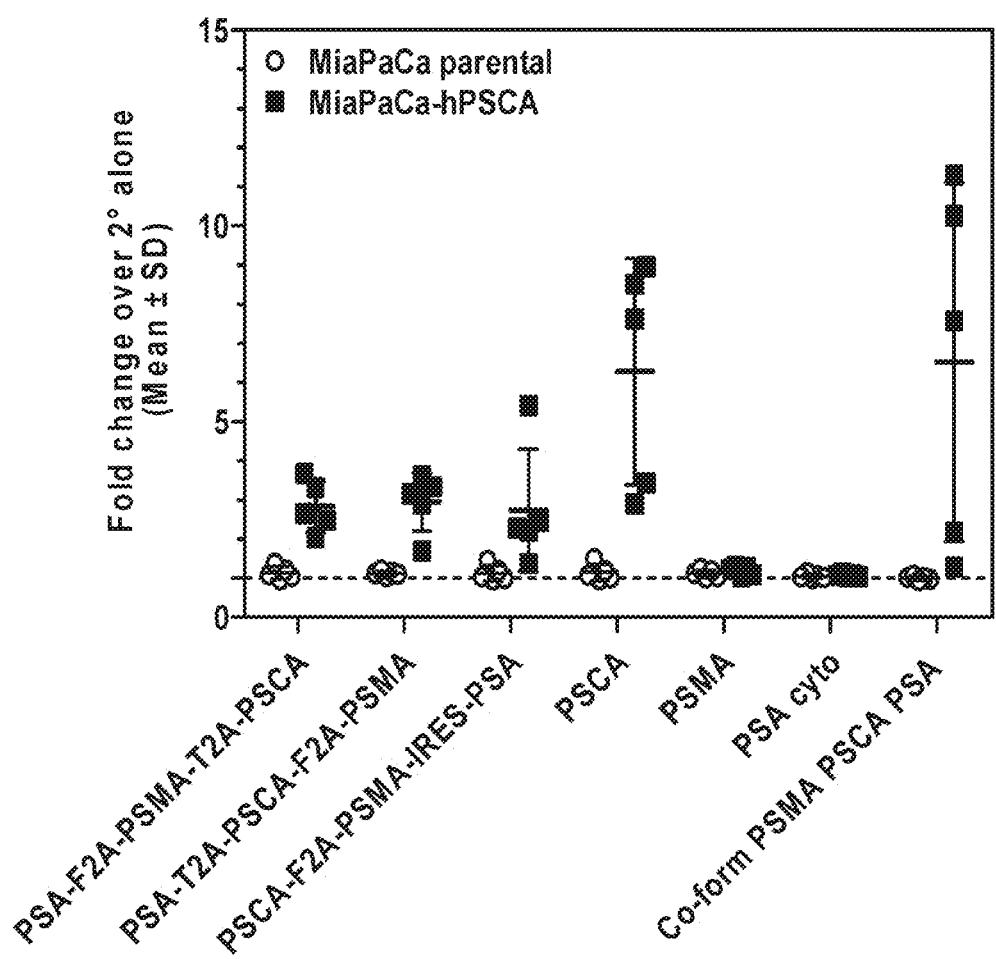
FIG. 14

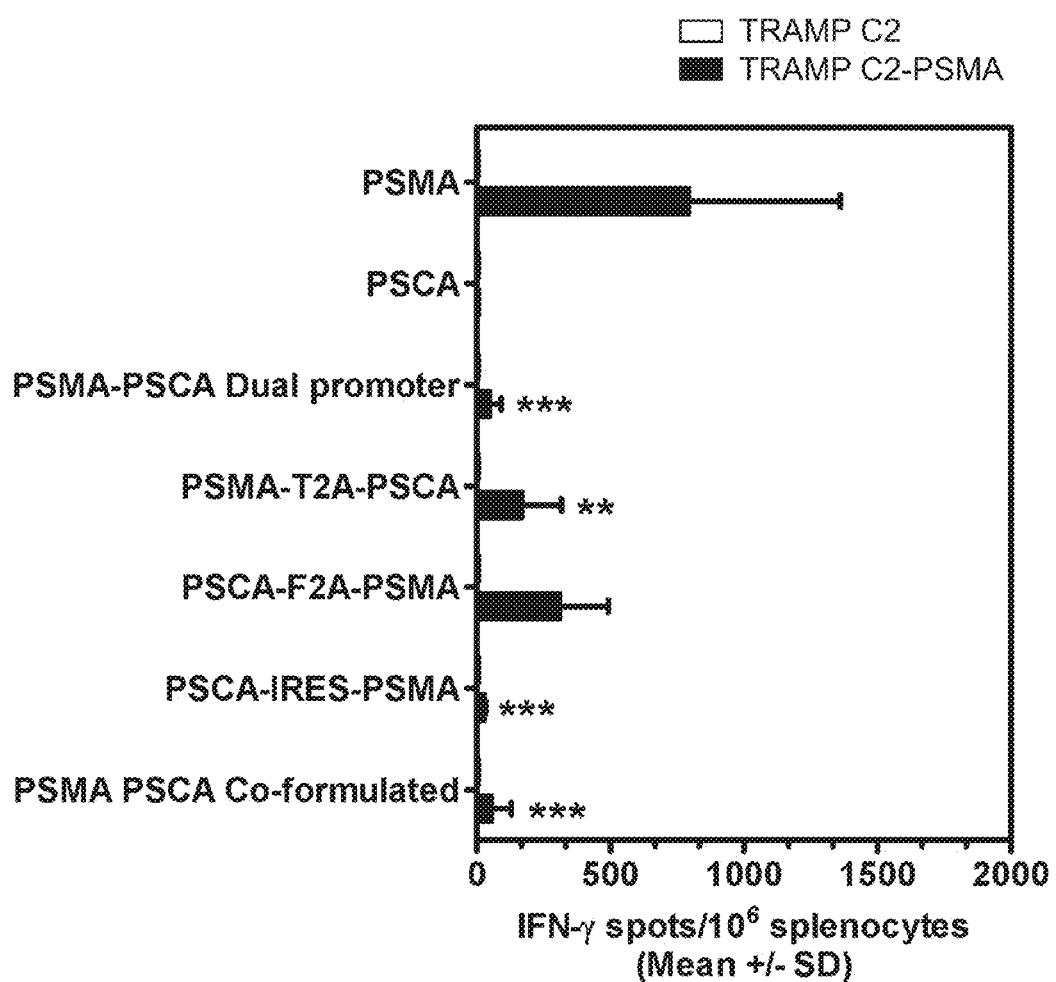
FIG. 15A

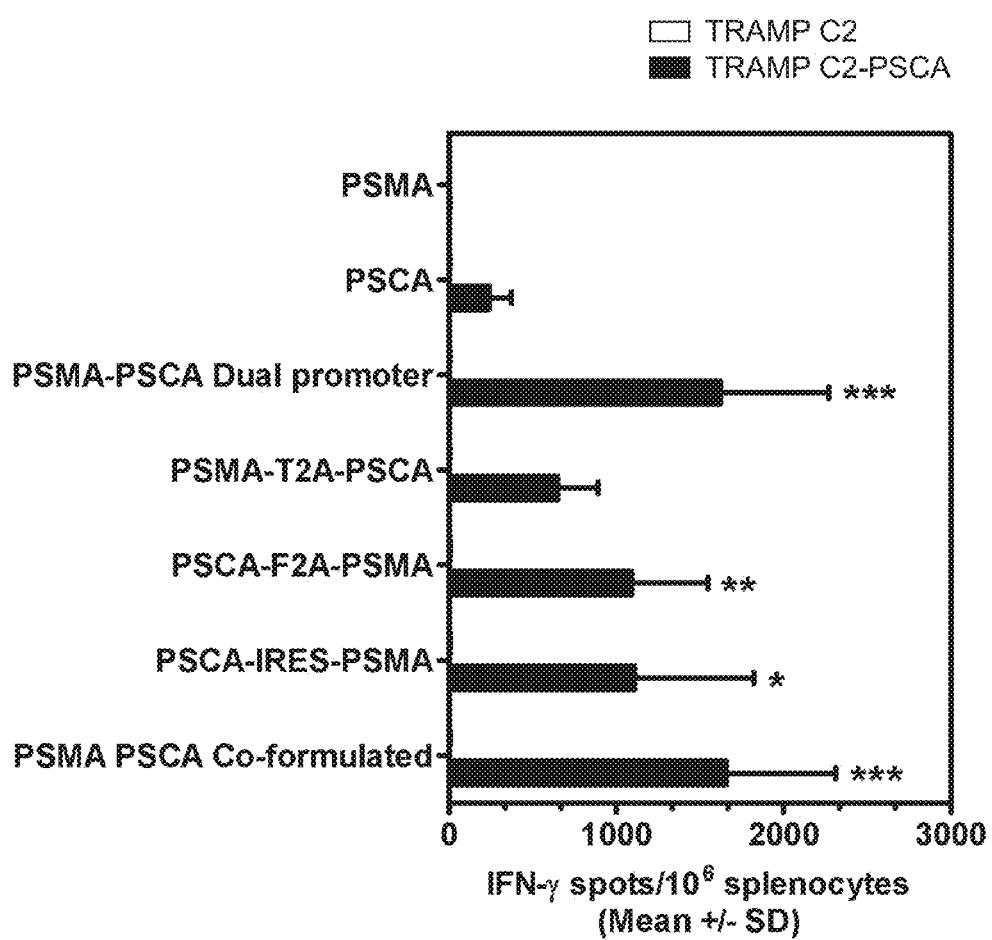
FIG. 15B

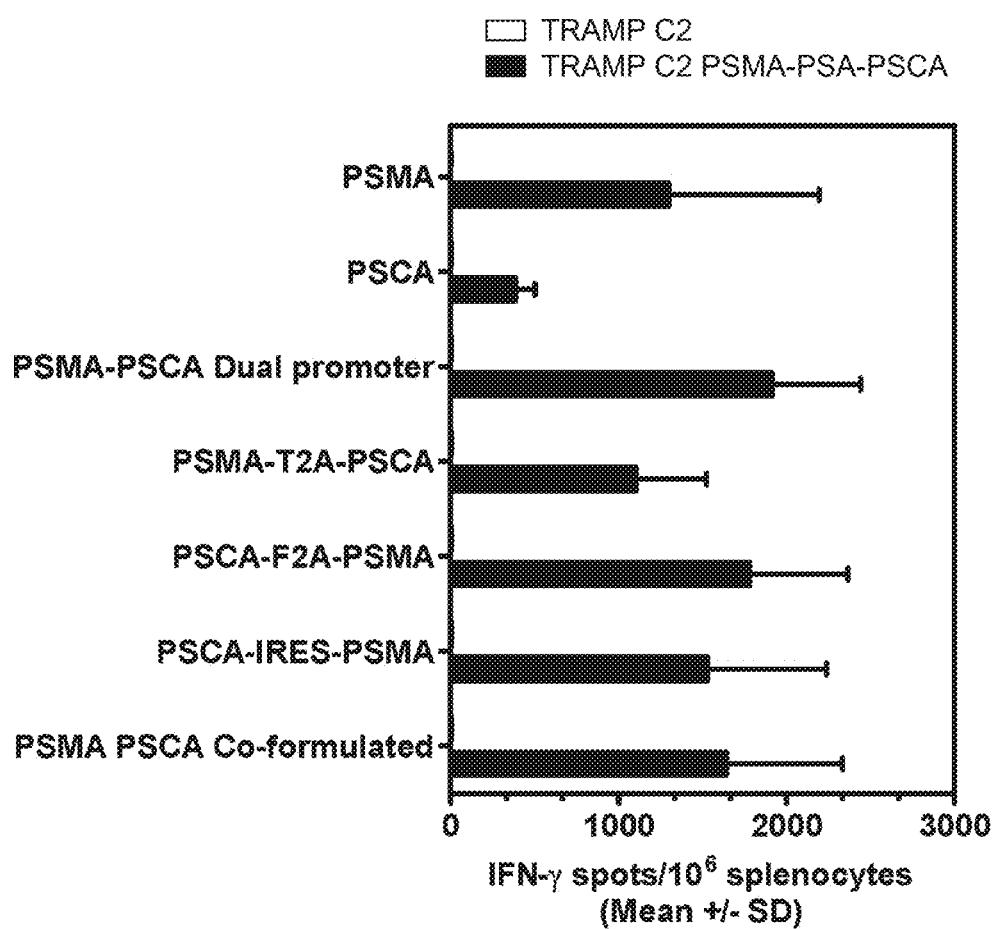
FIG. 15C

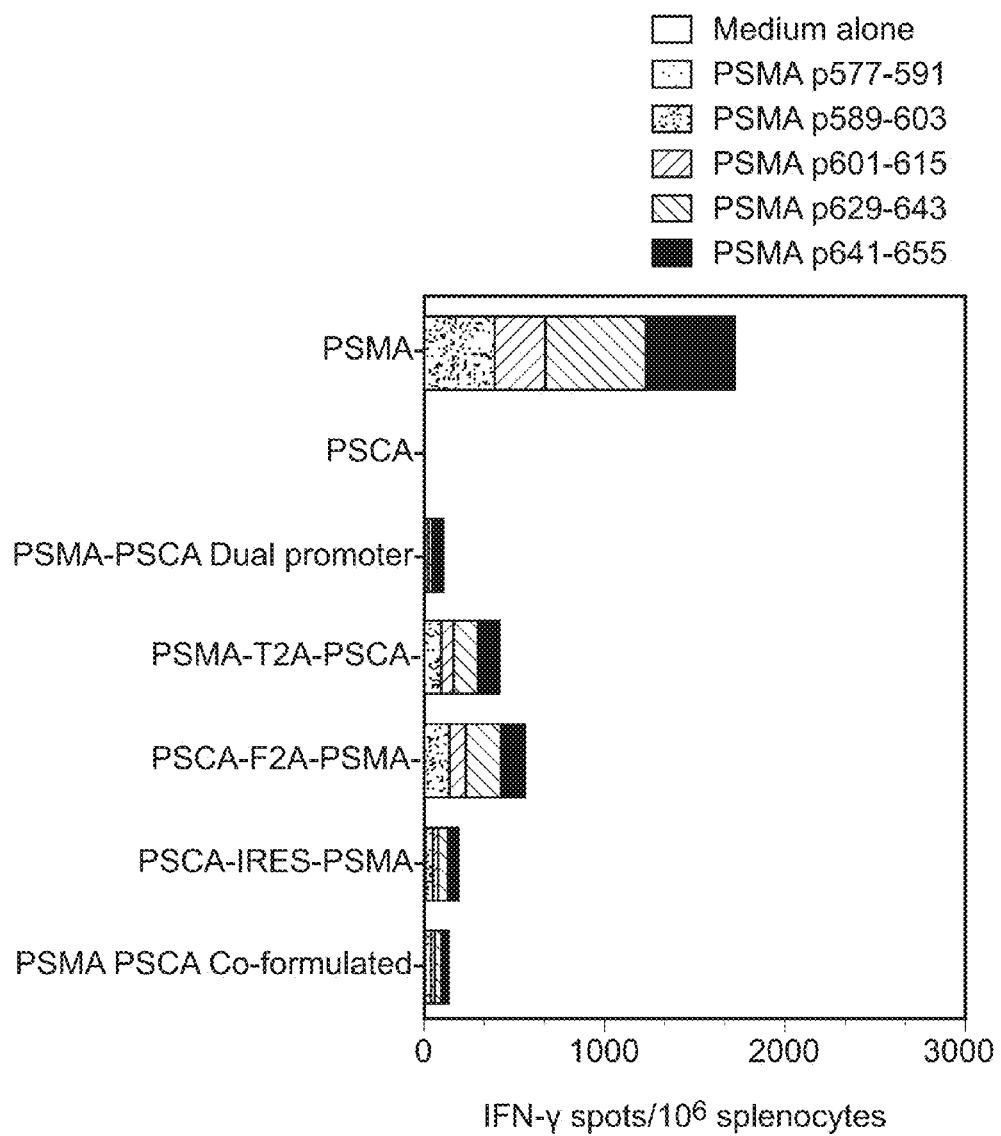
FIG. 16A

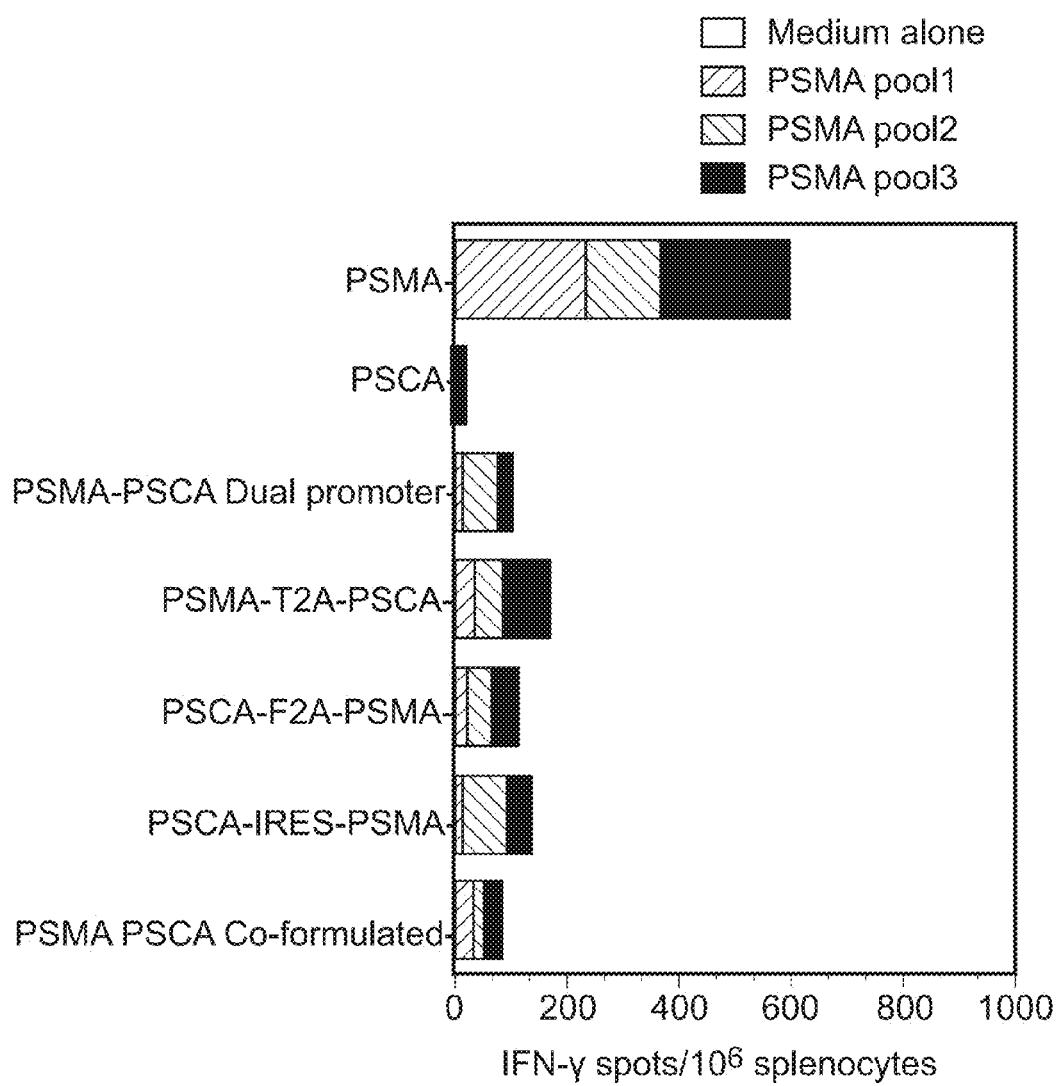
FIG. 16B

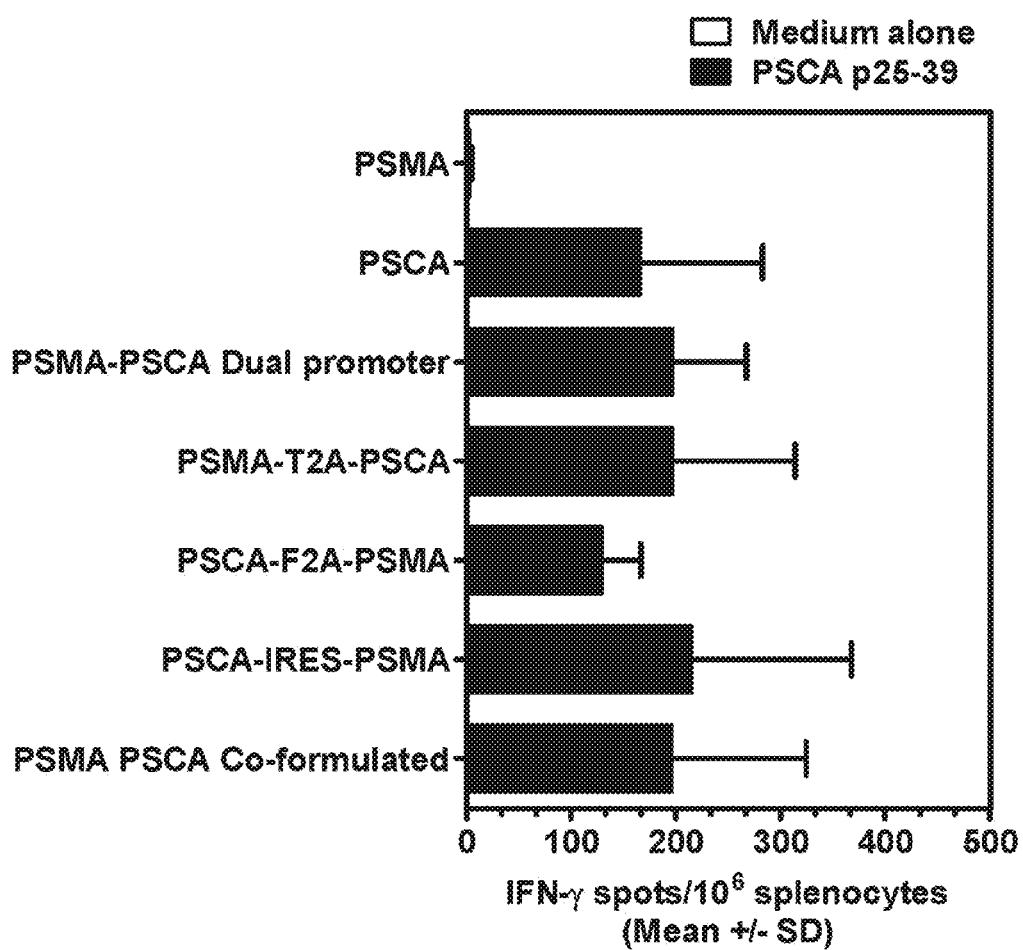
FIG. 16C

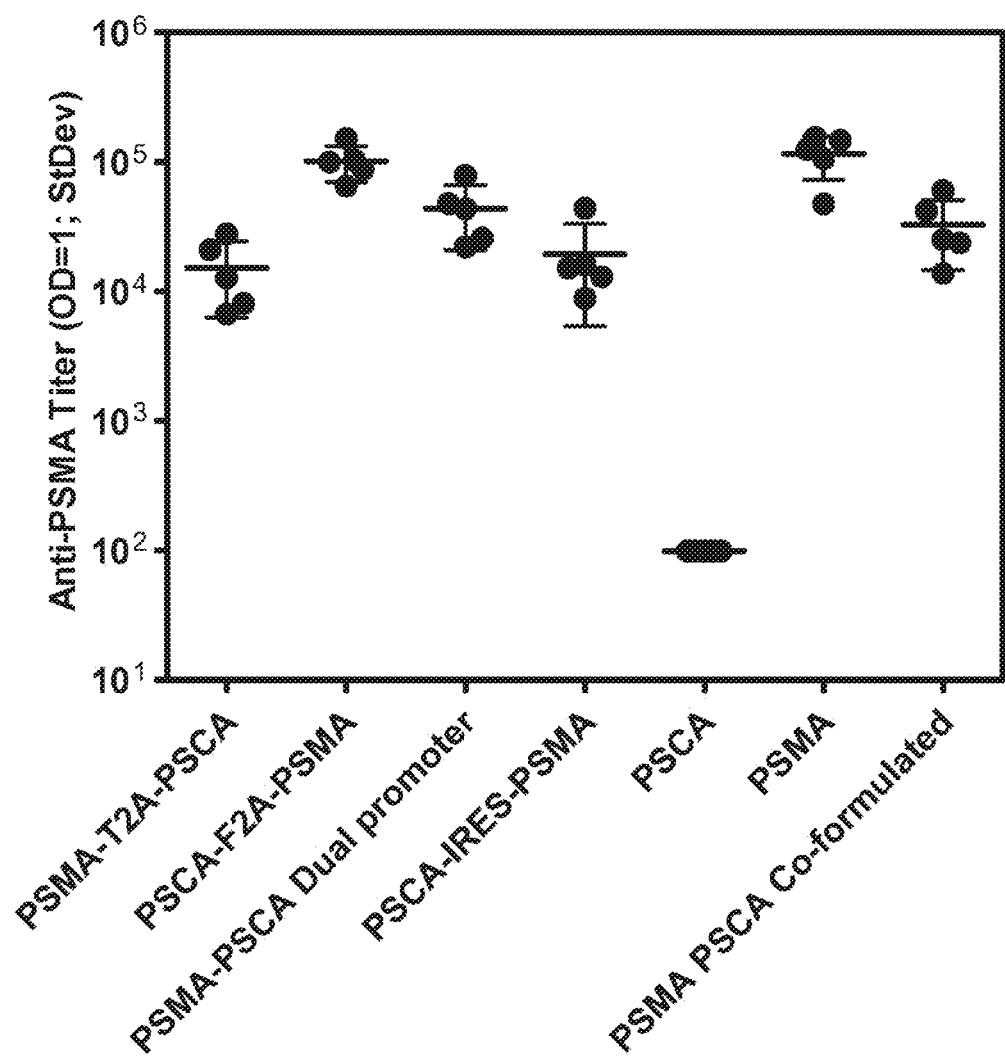
FIG. 17

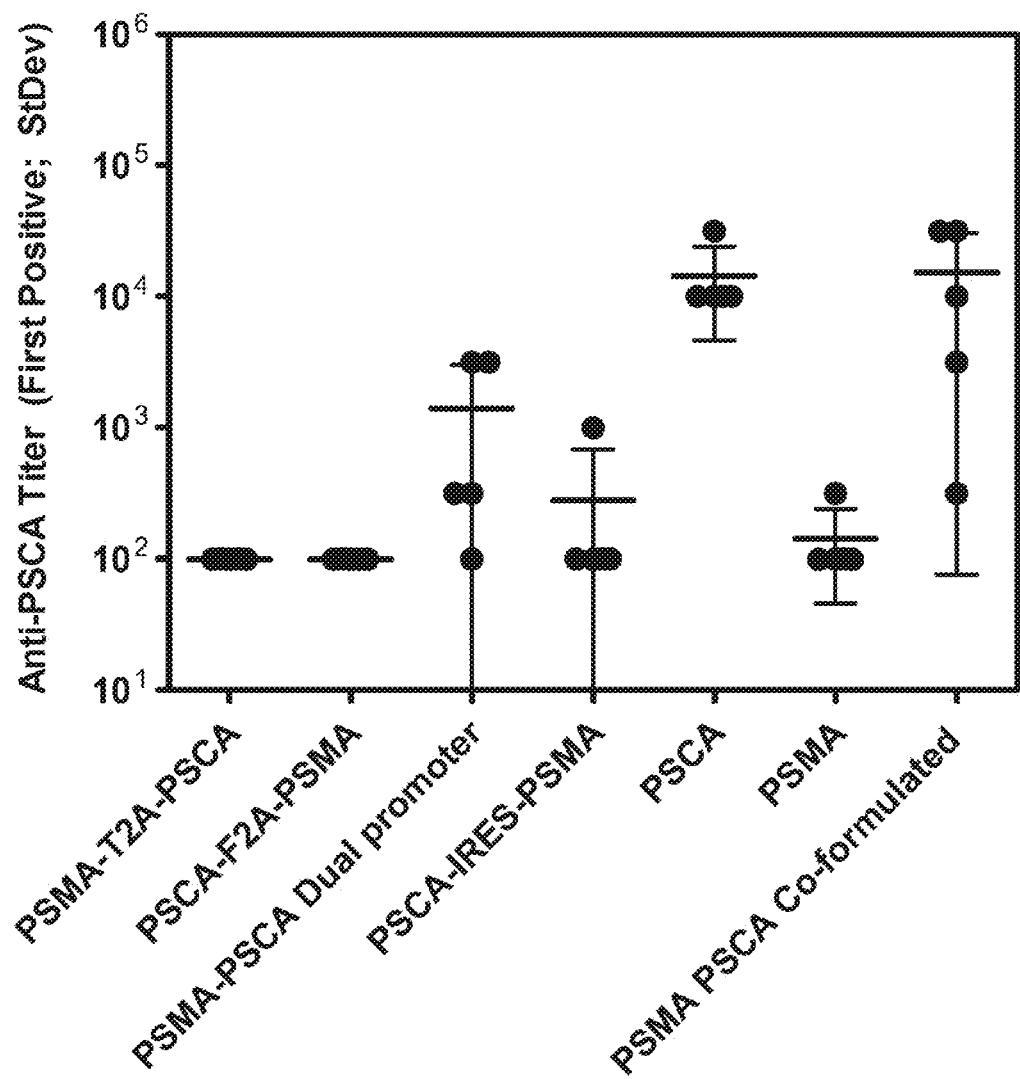
FIG. 18

FIG. 19

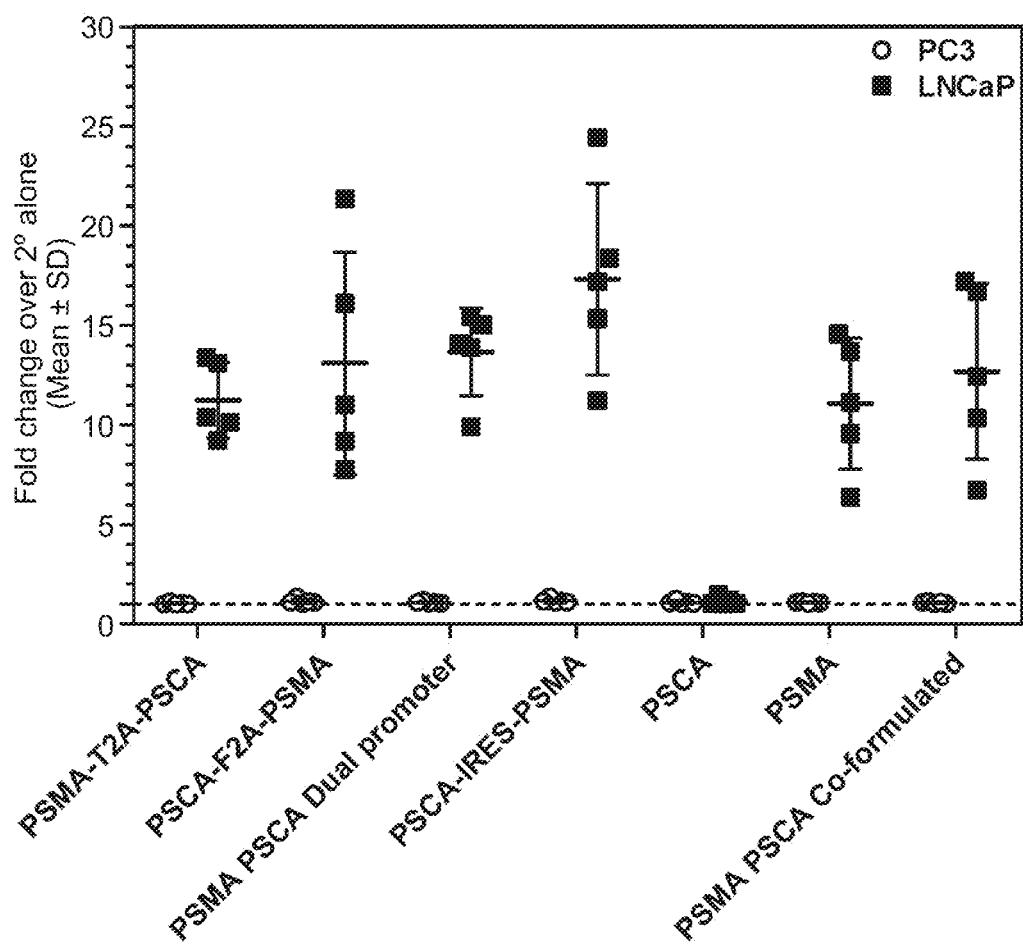


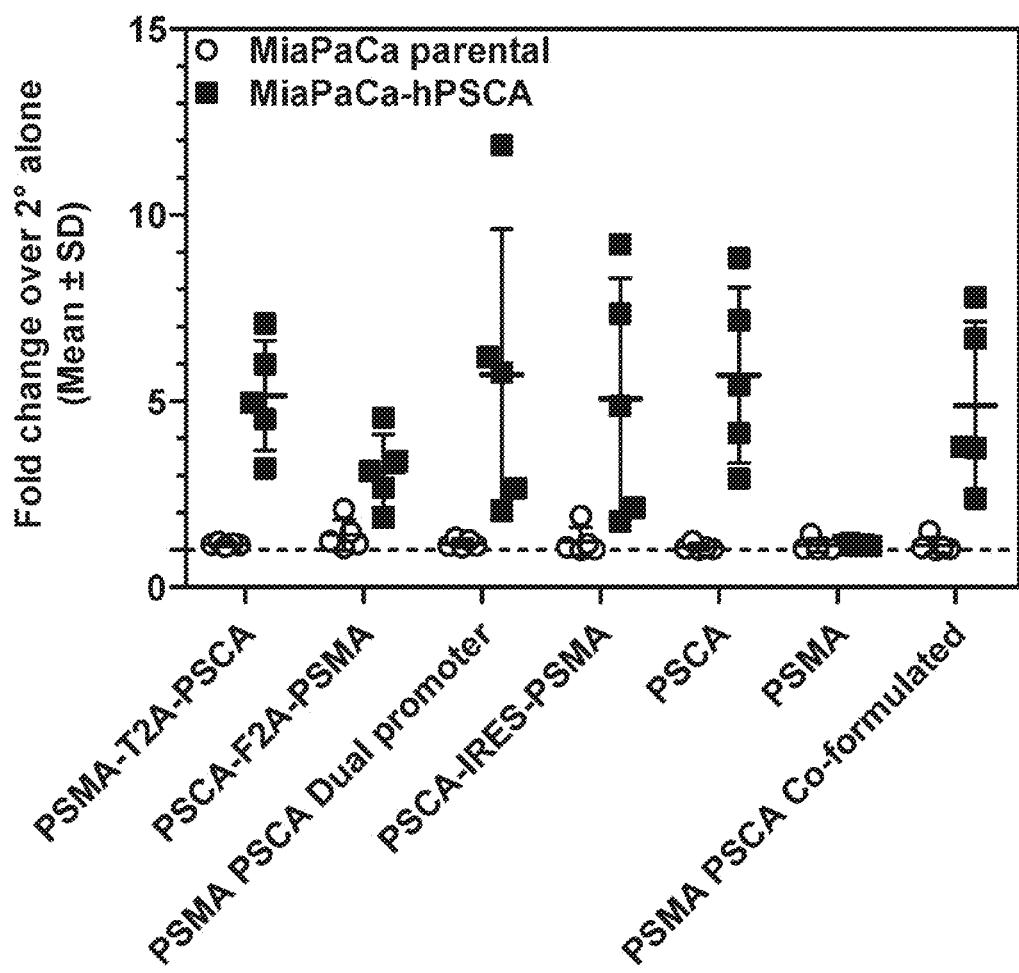
FIG. 20

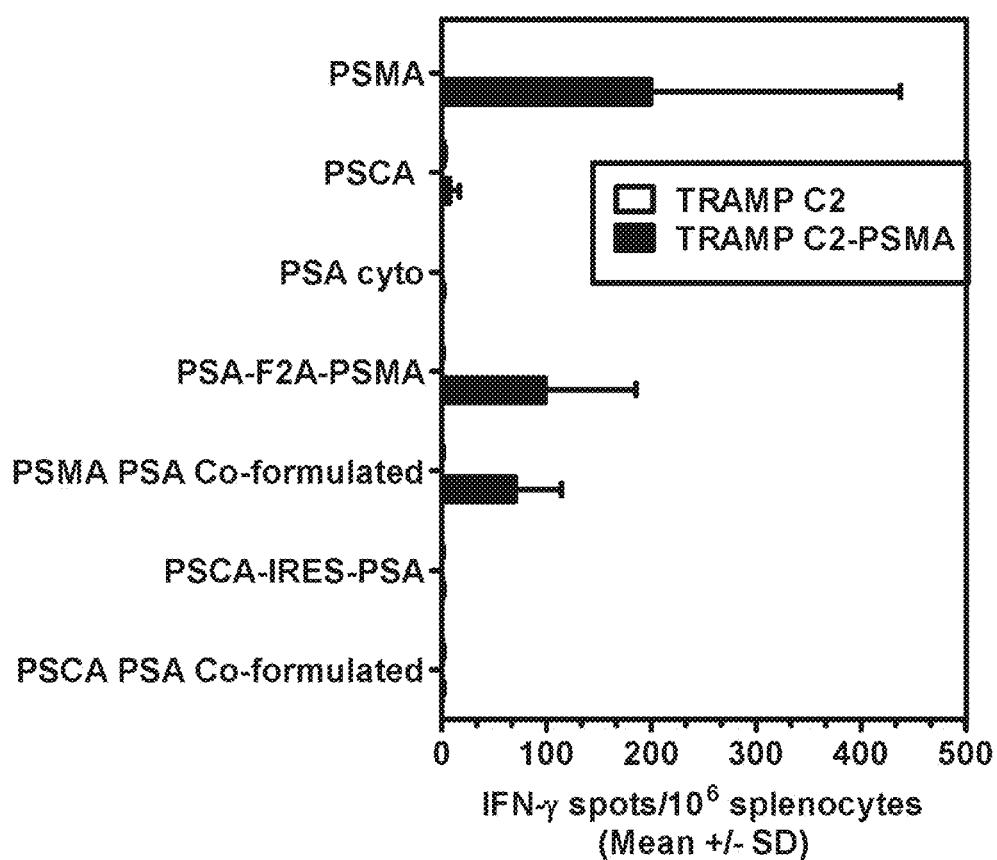
FIG. 21A

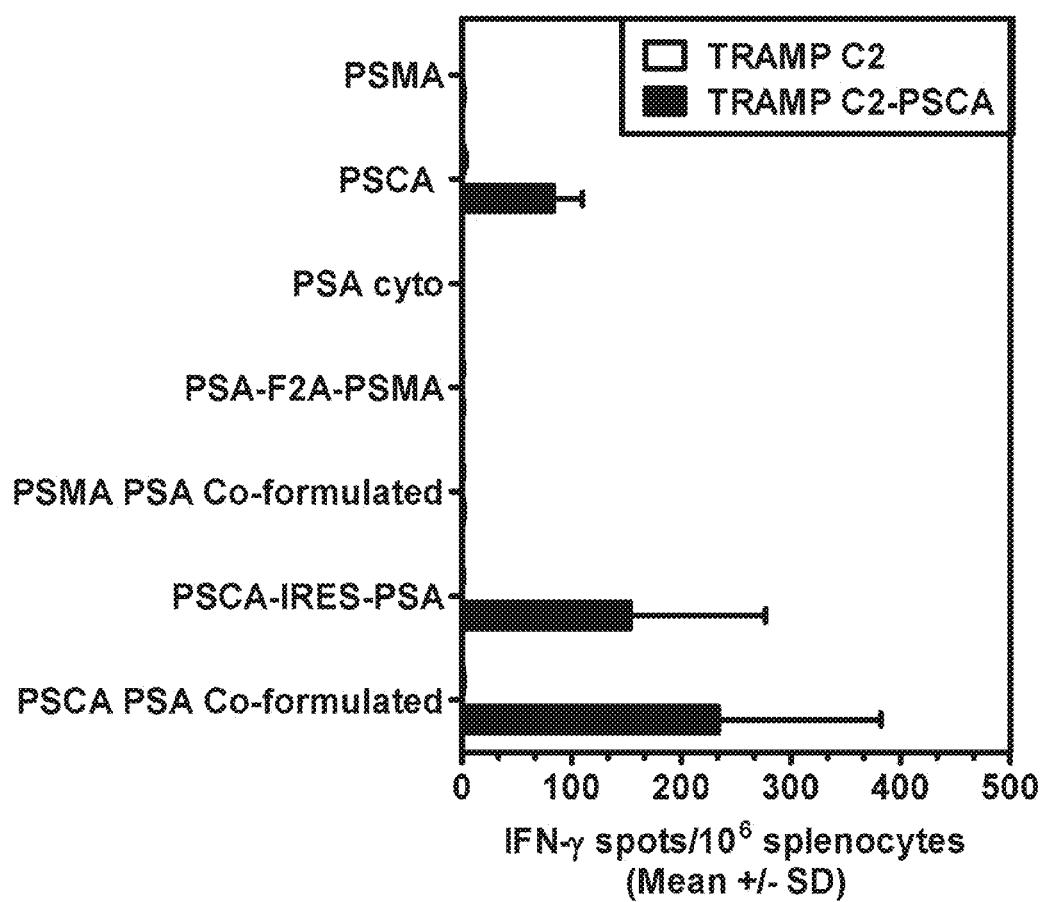
FIG. 21B

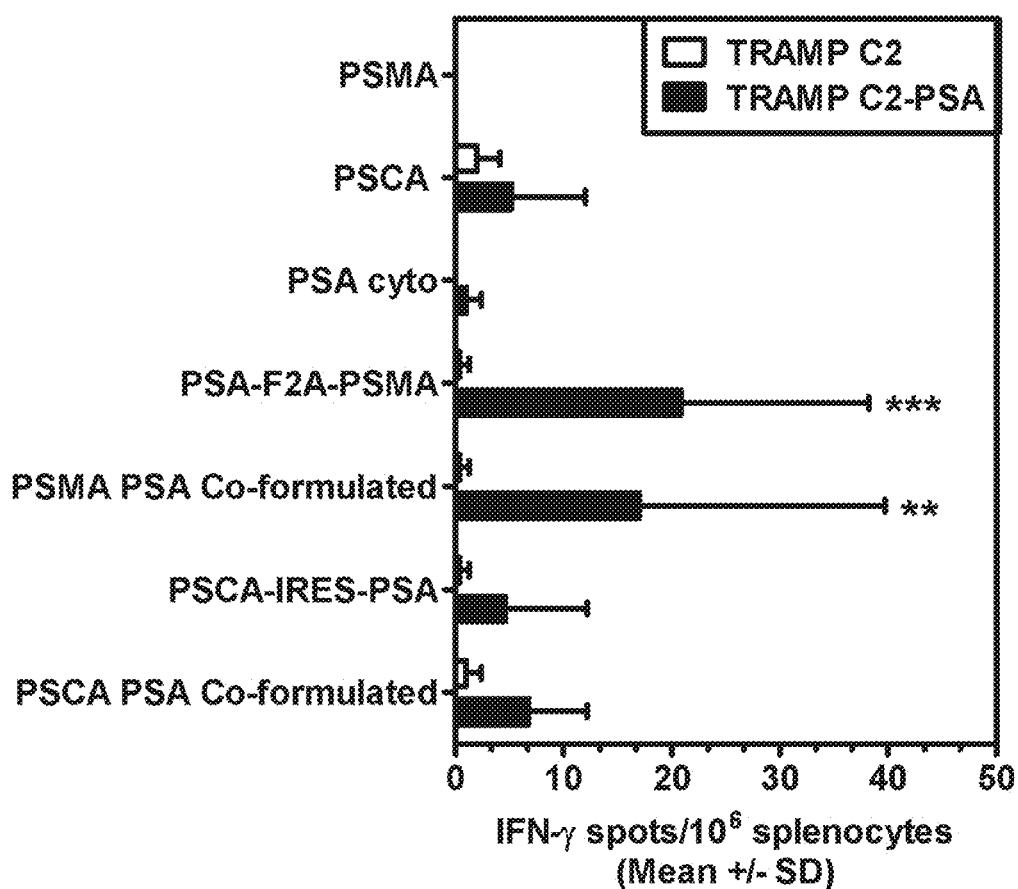
FIG. 21C

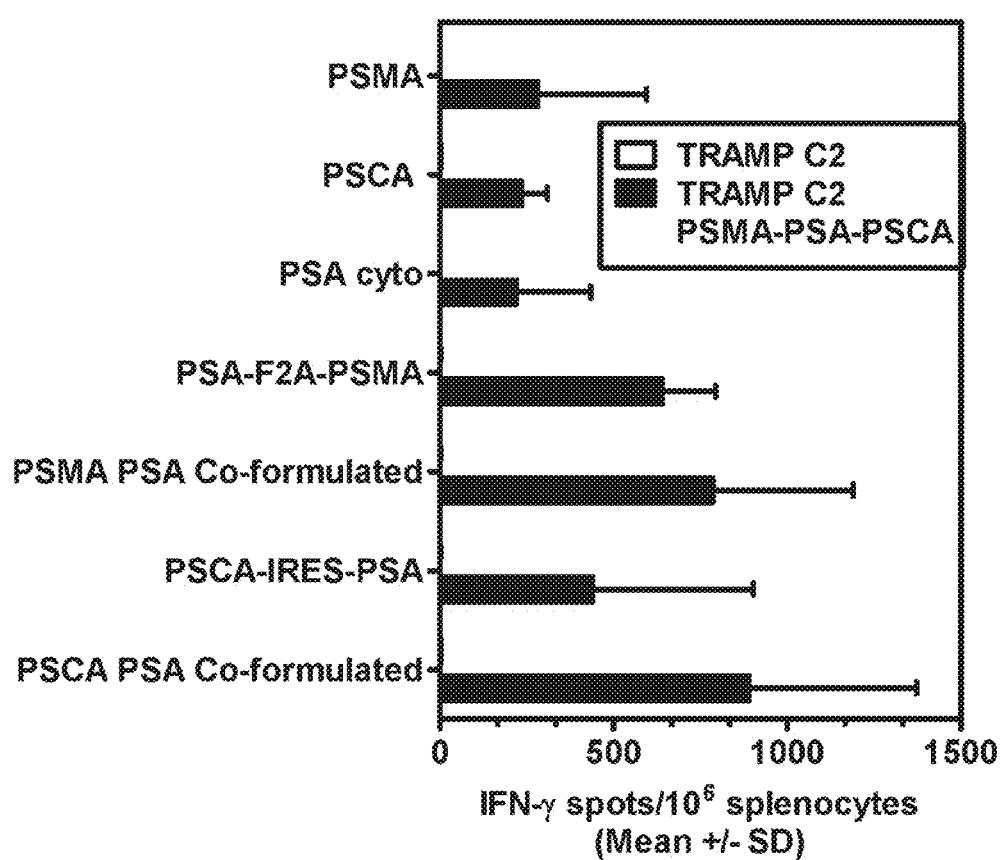
FIG. 21D

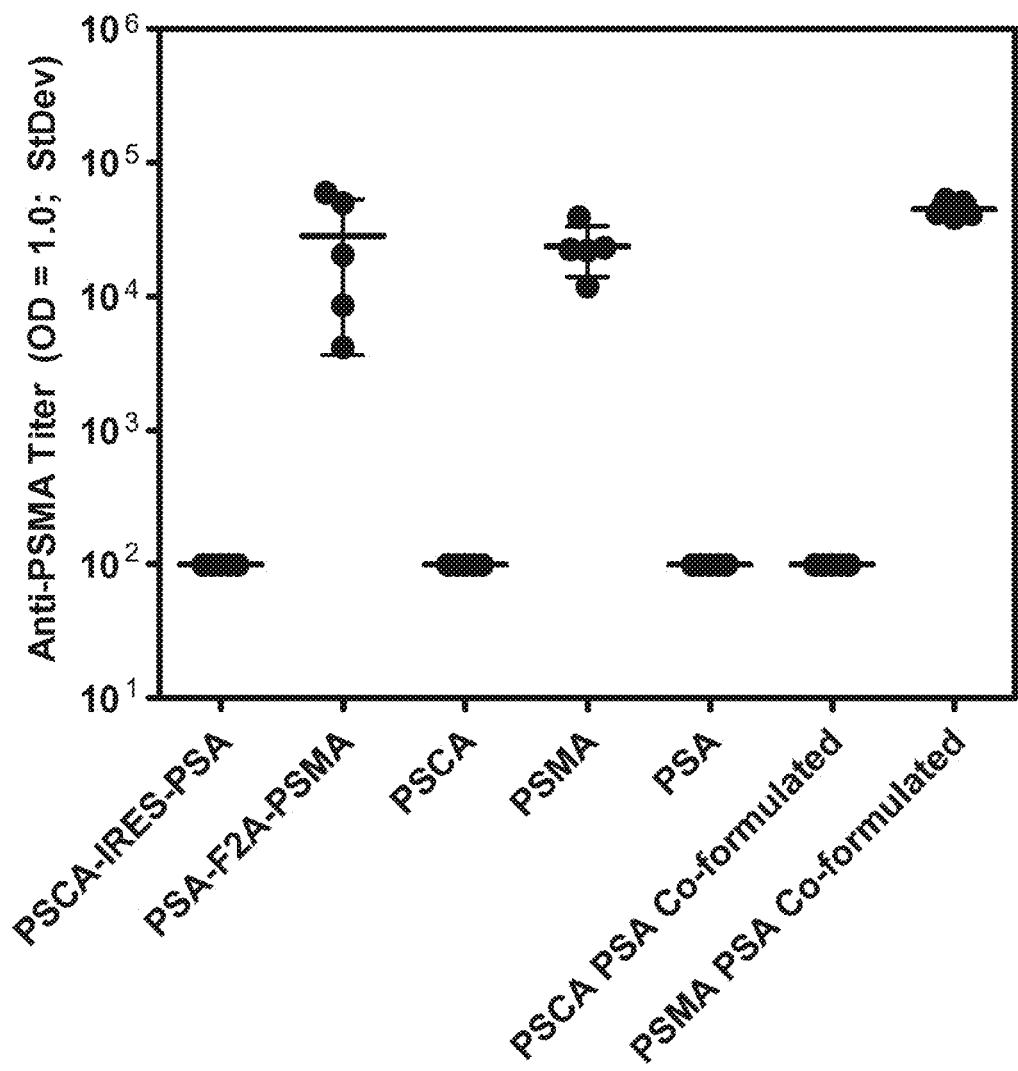
FIG. 22

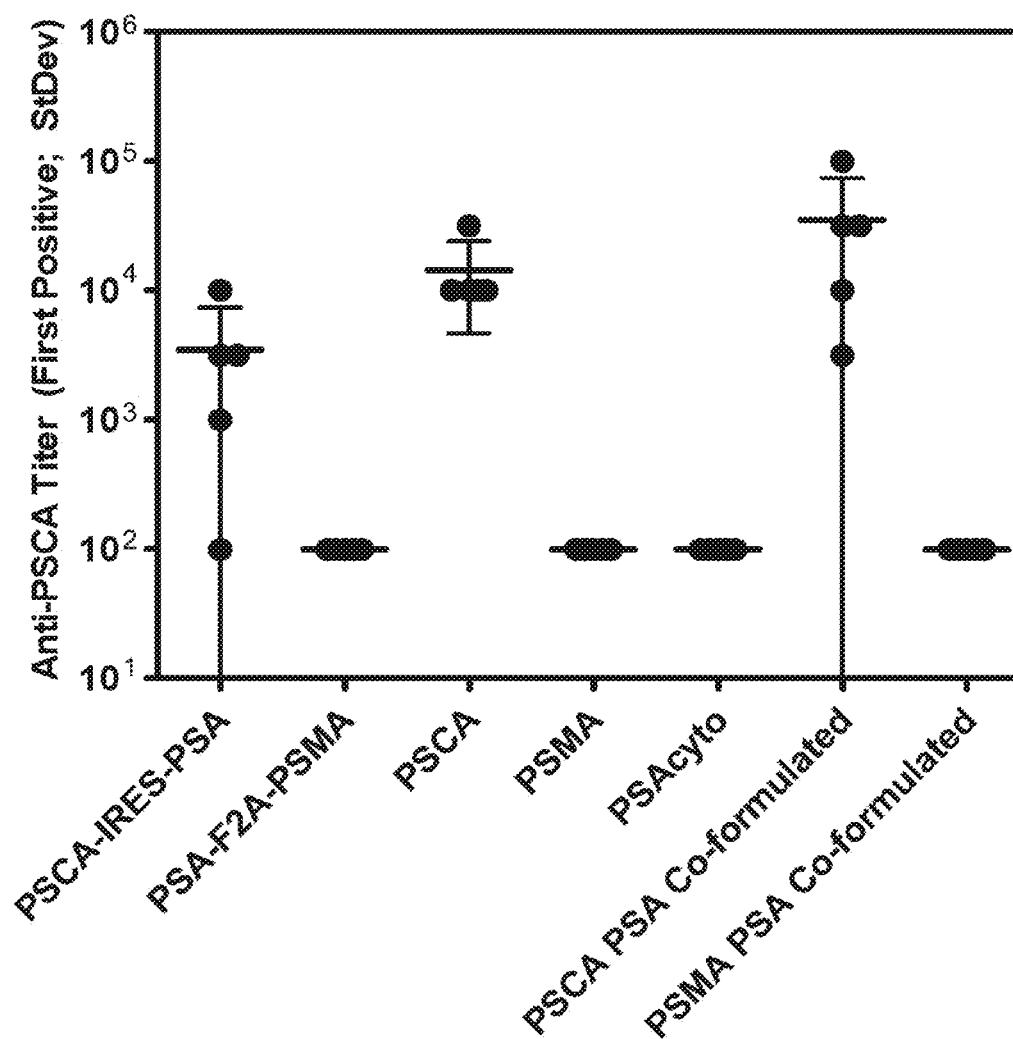
FIG. 23

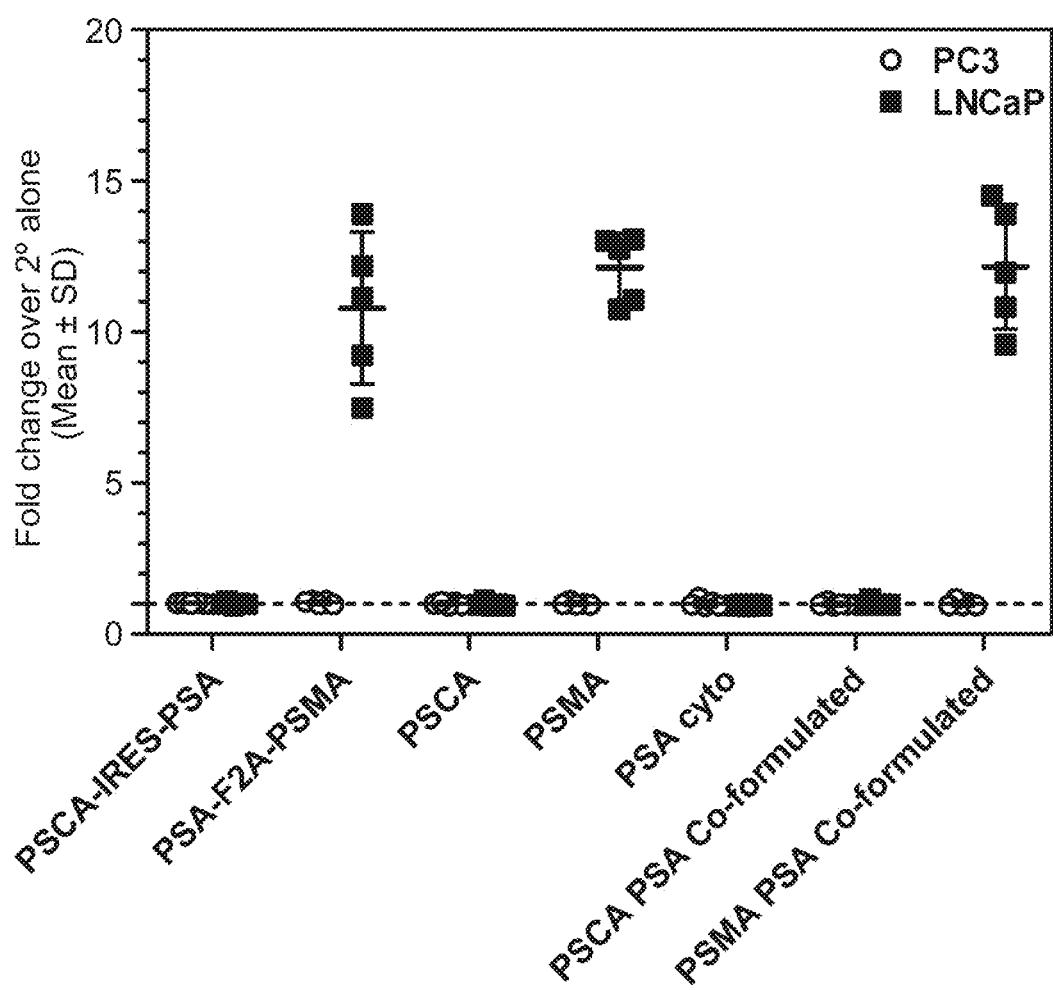
FIG. 24

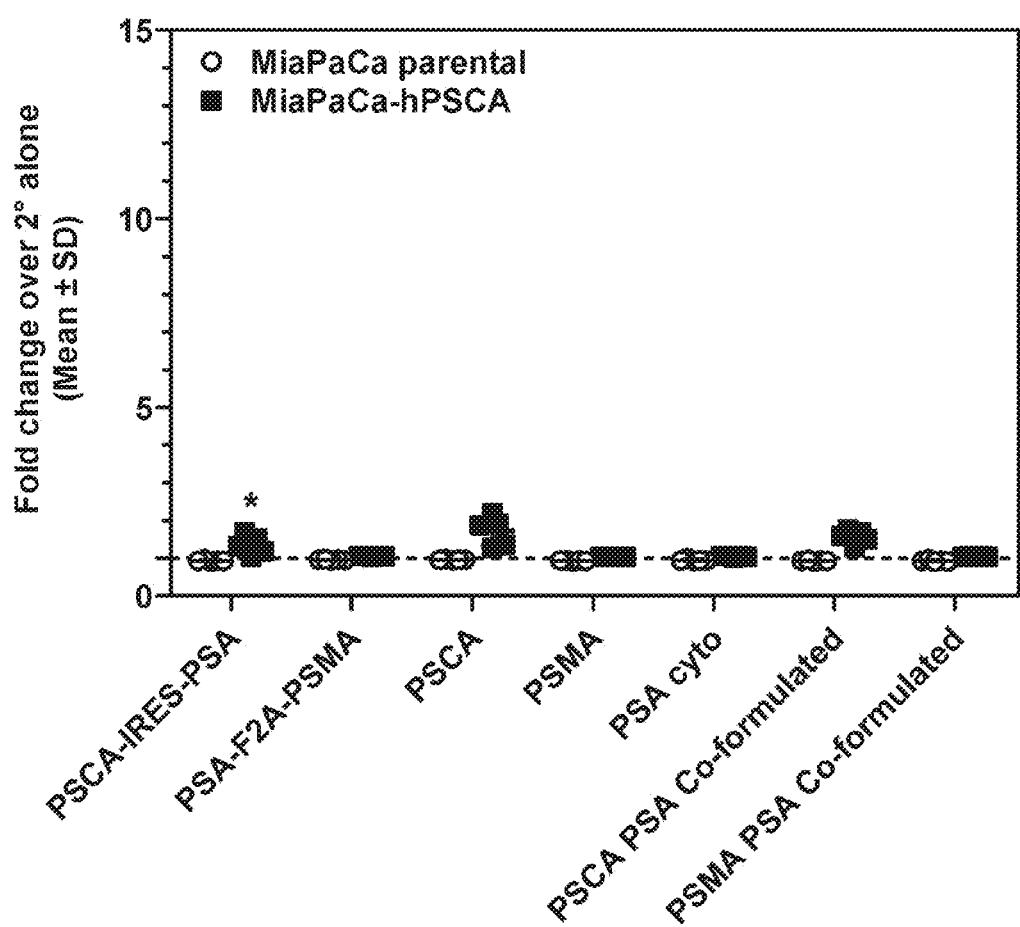
FIG. 25

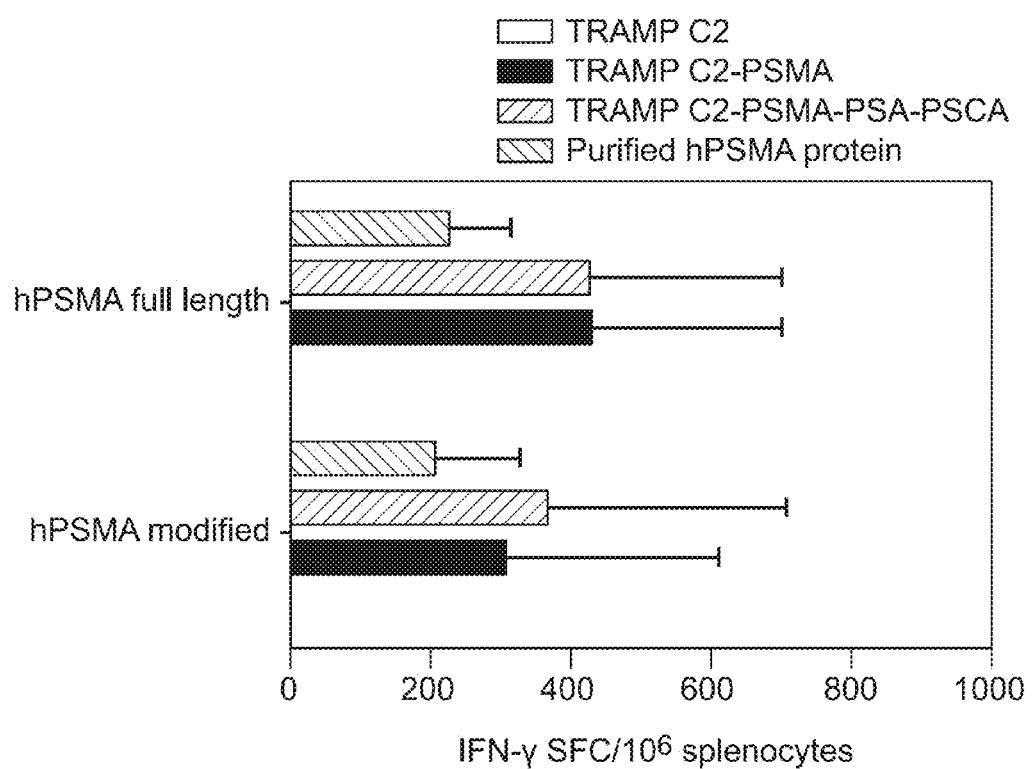
FIG. 26

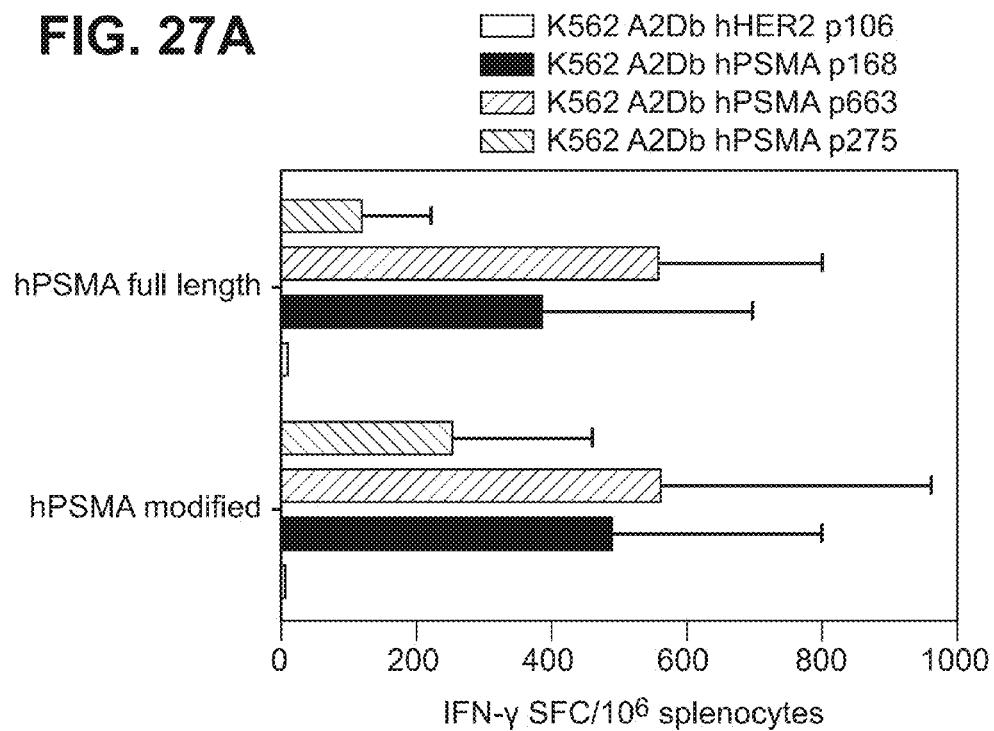
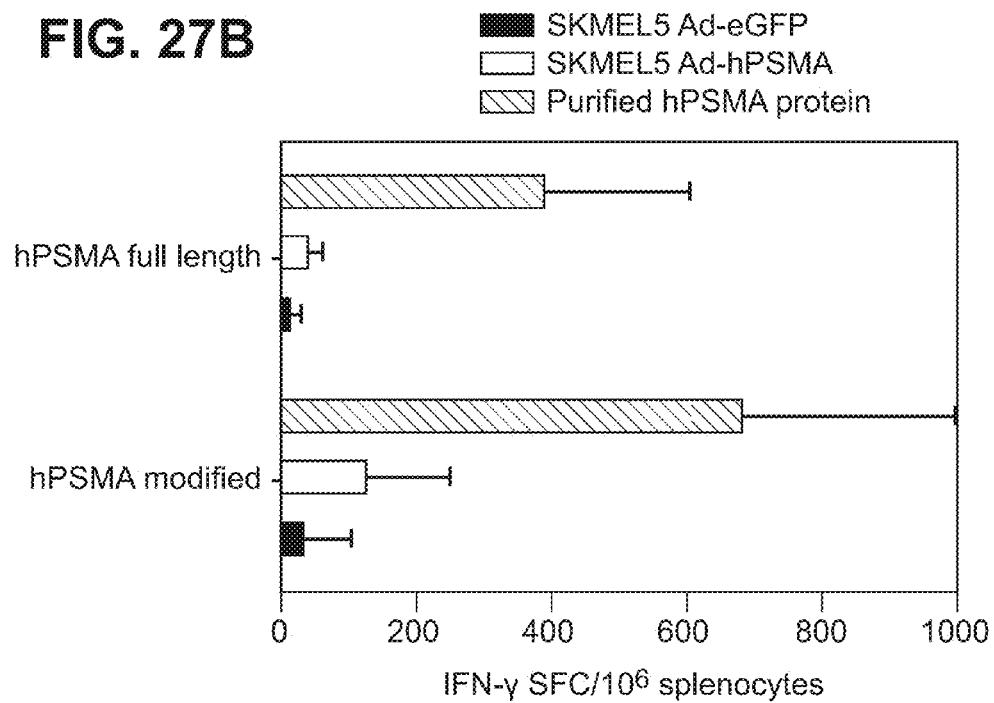
FIG. 27A**FIG. 27B**

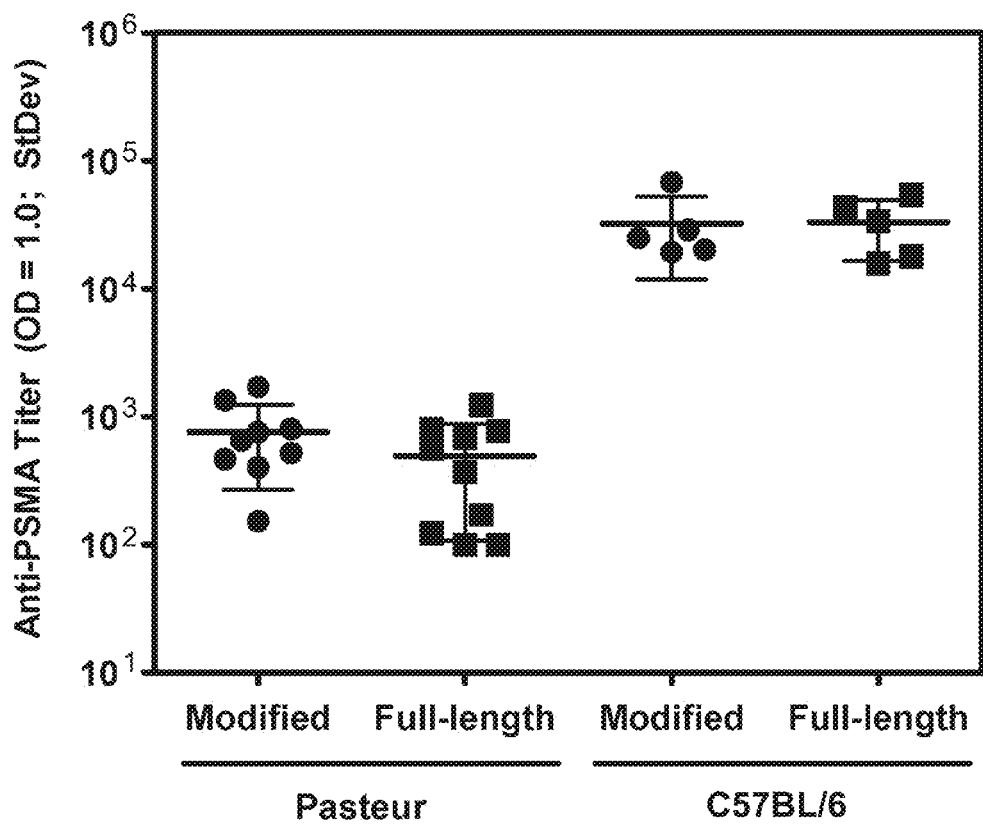
FIG. 28

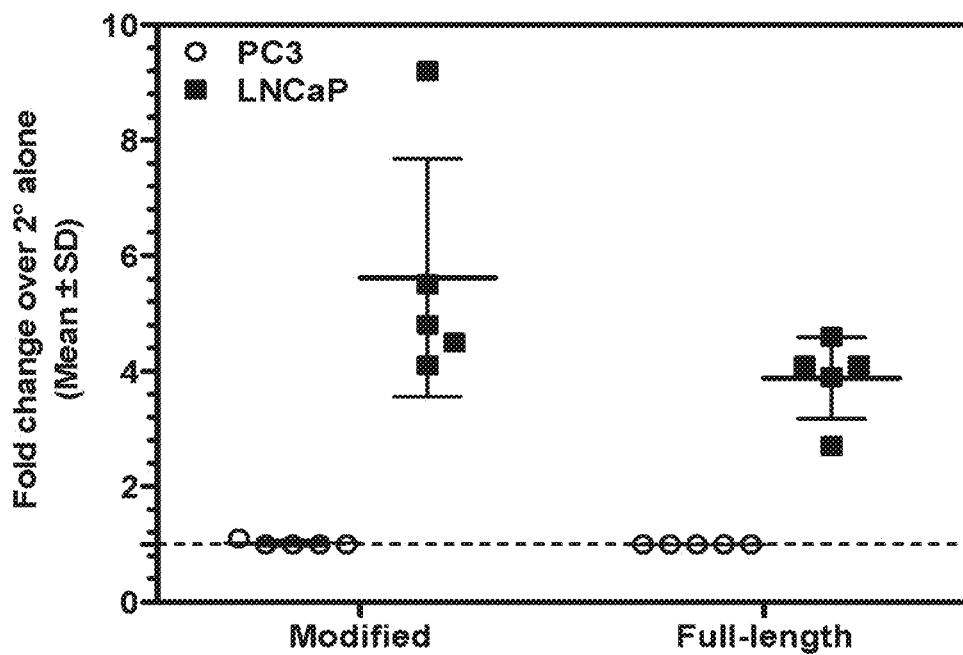
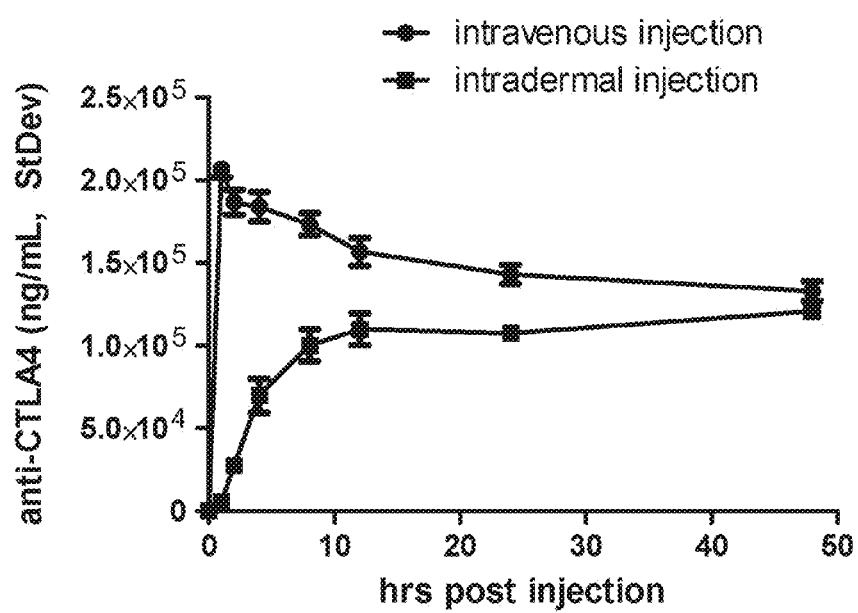
FIG. 29**FIG. 30**

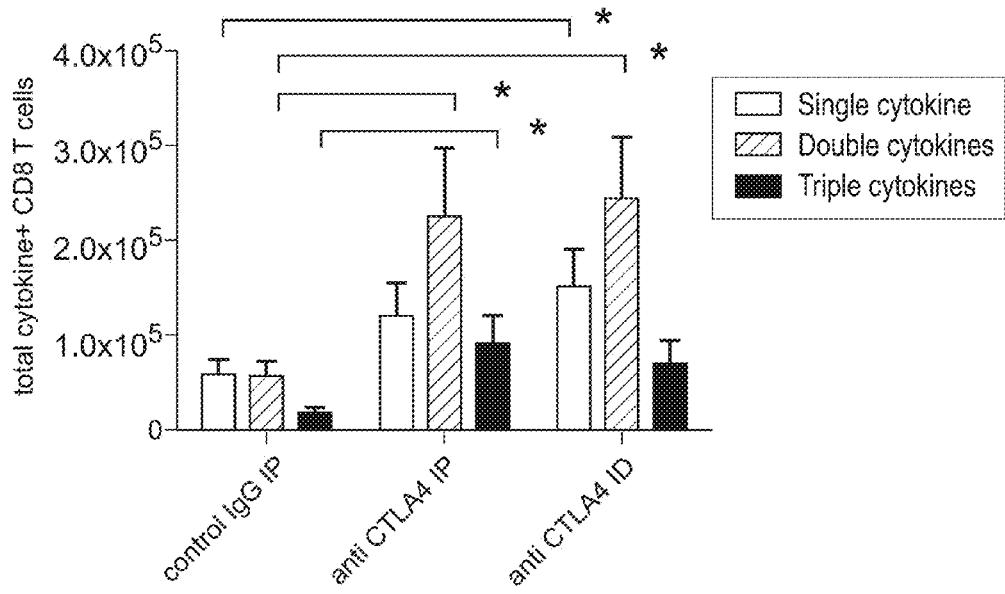
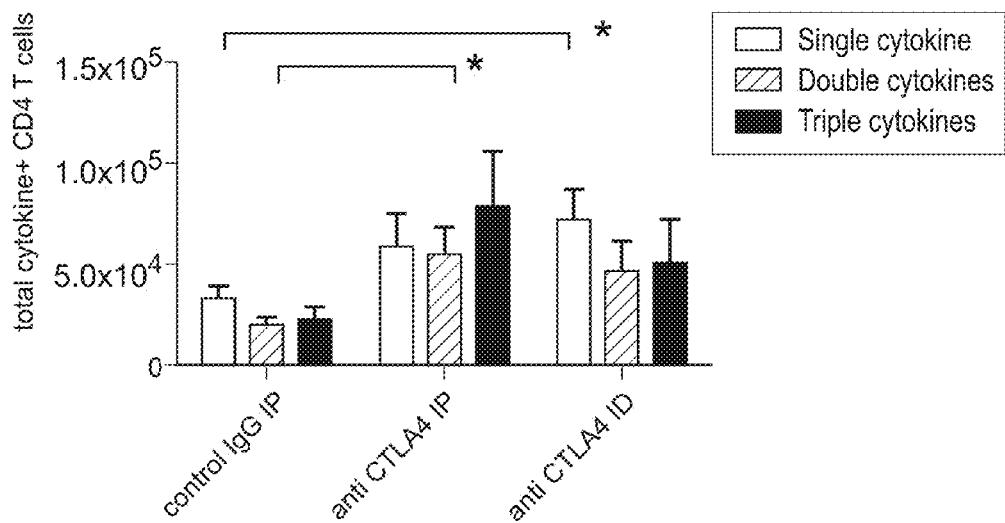
FIG. 31A rHer-2 specific polyfunctional CD8 T cells**FIG. 31B** rHer-2 specific polyfunctional CD4 T cells

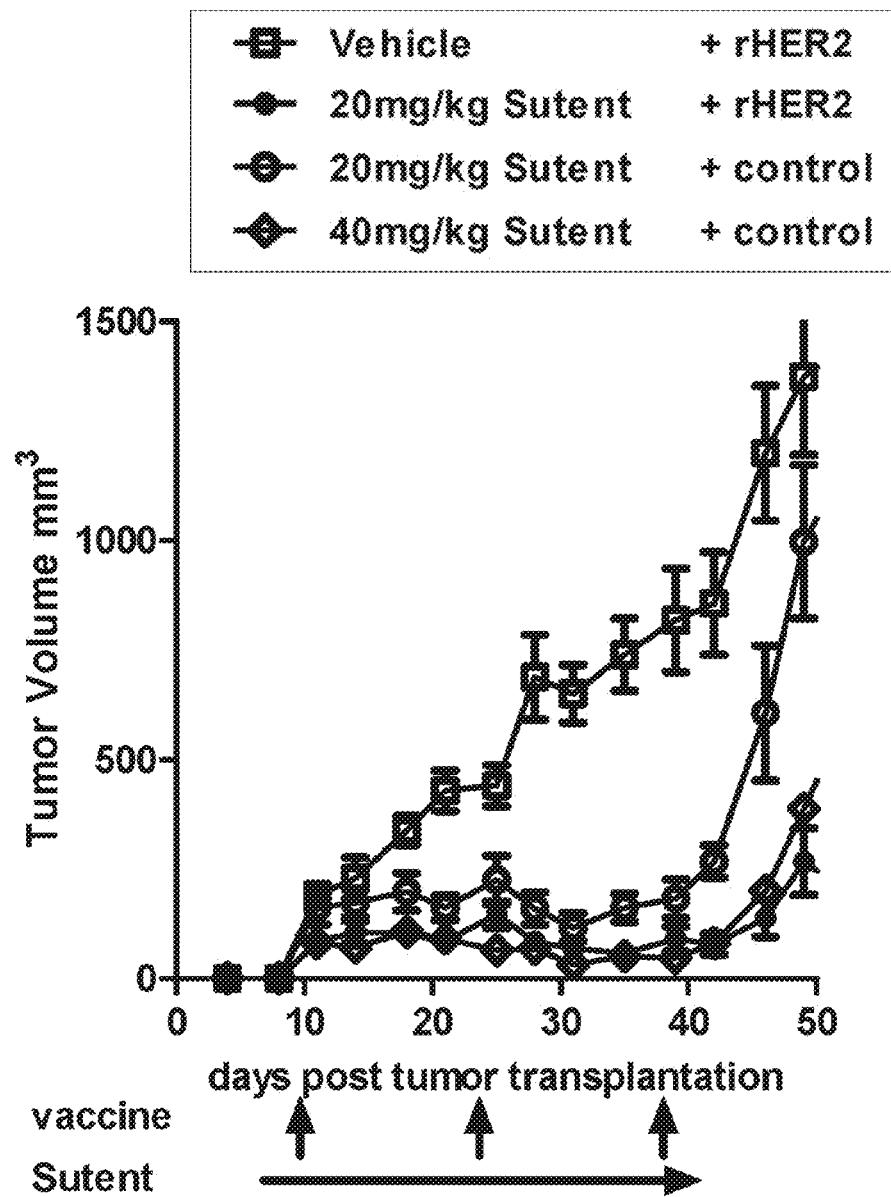
FIG. 32

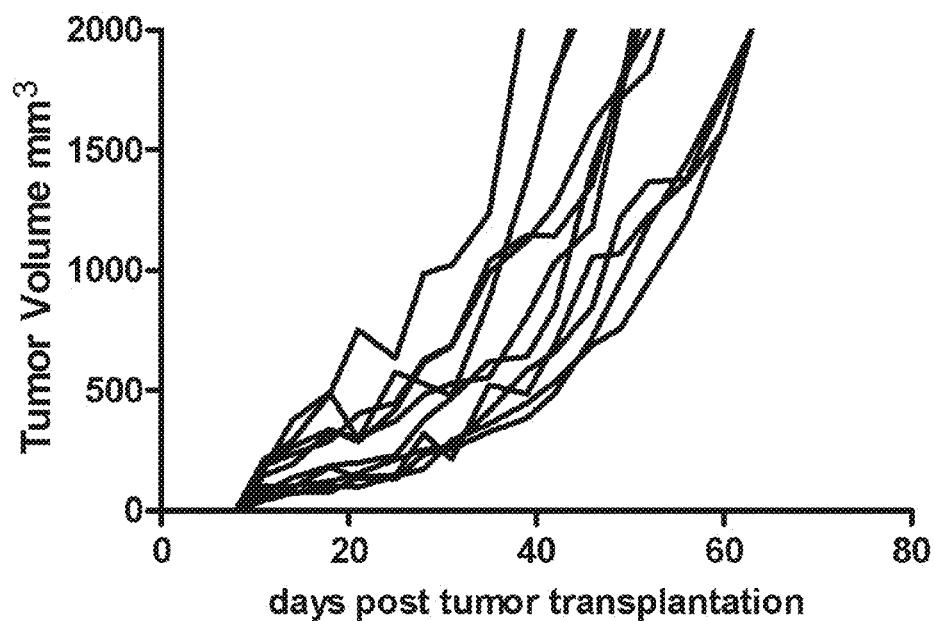
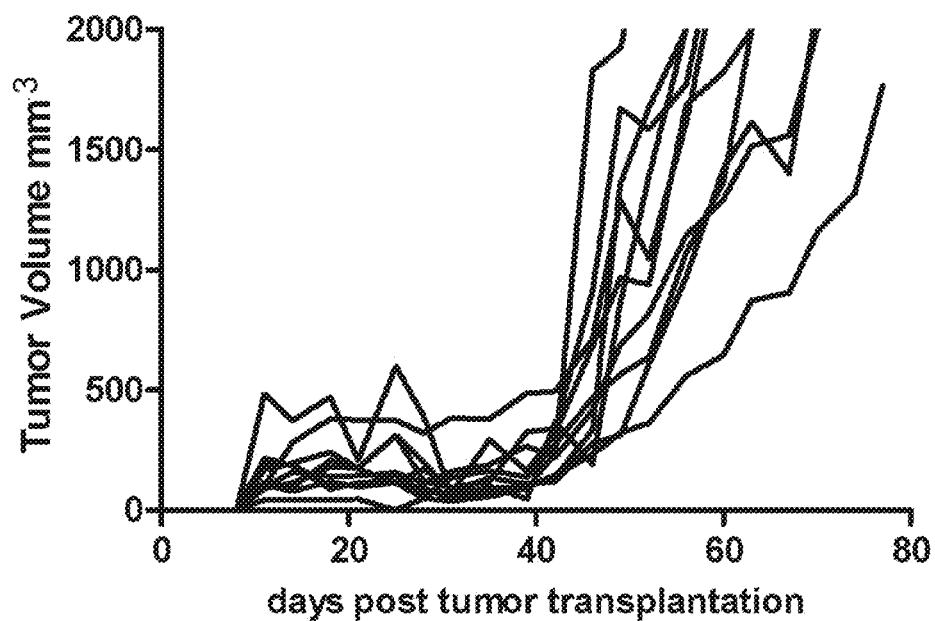
FIG. 33A — vehicle + control**FIG. 33B** — Sutent + Control

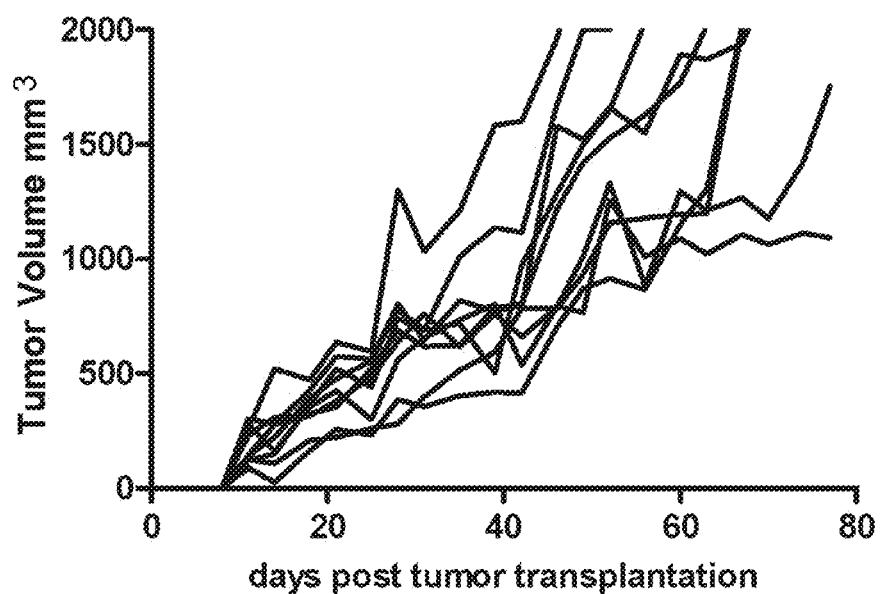
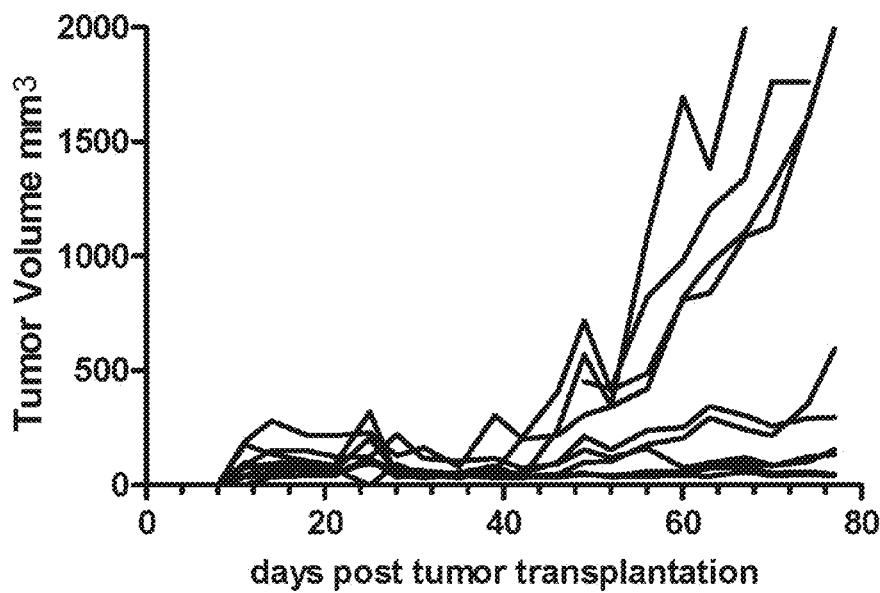
FIG. 33C — vehicle + rHER2**FIG. 33D** — Sutent + rHER2

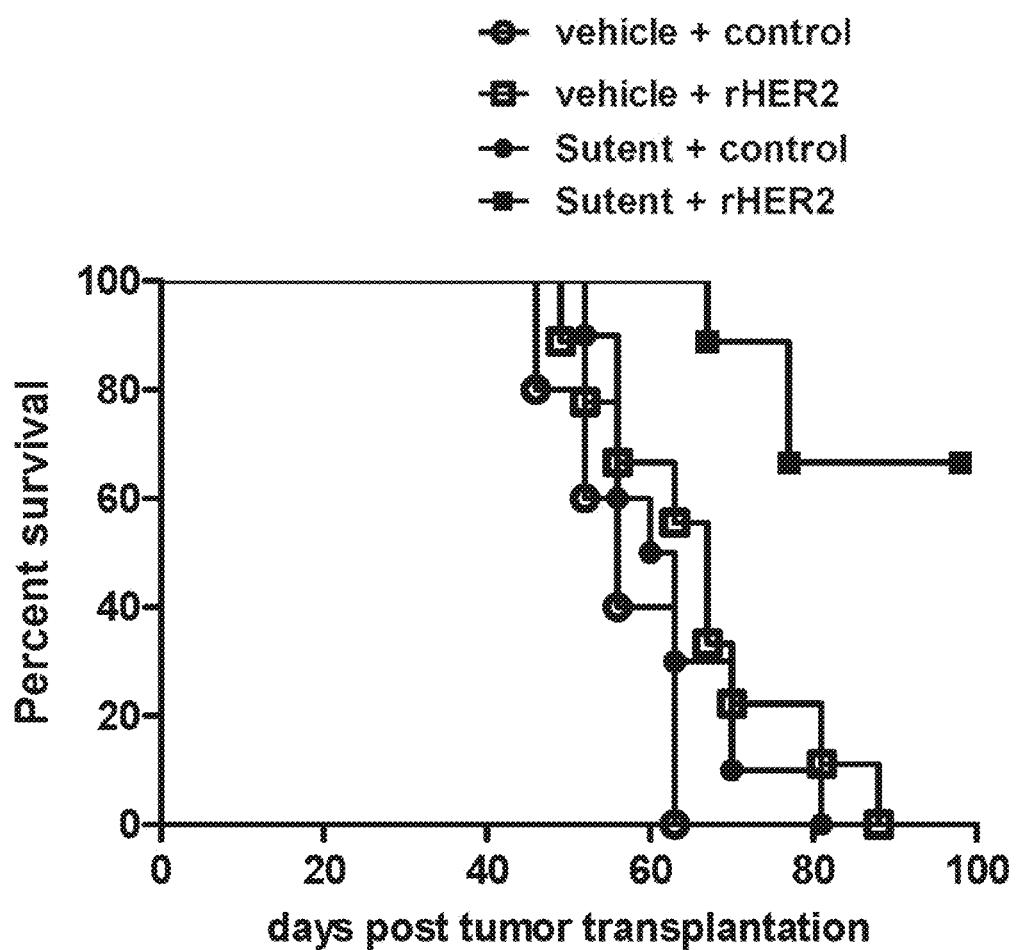
FIG. 34

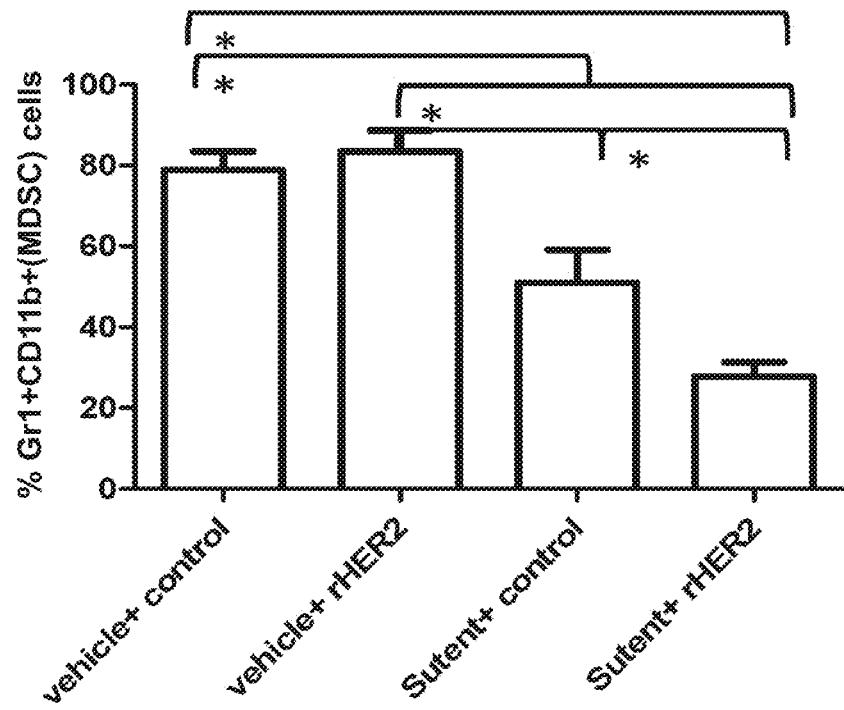
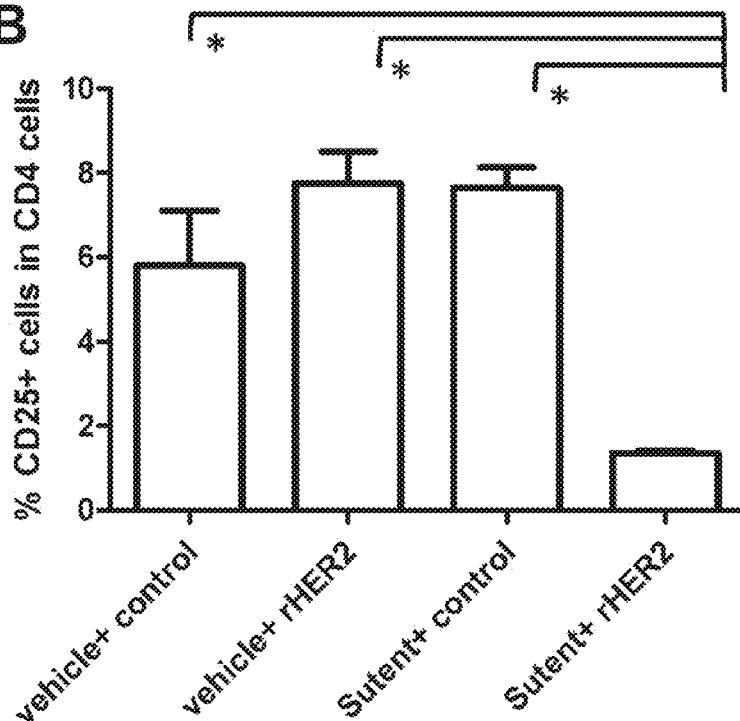
FIG. 35A**FIG. 35B**

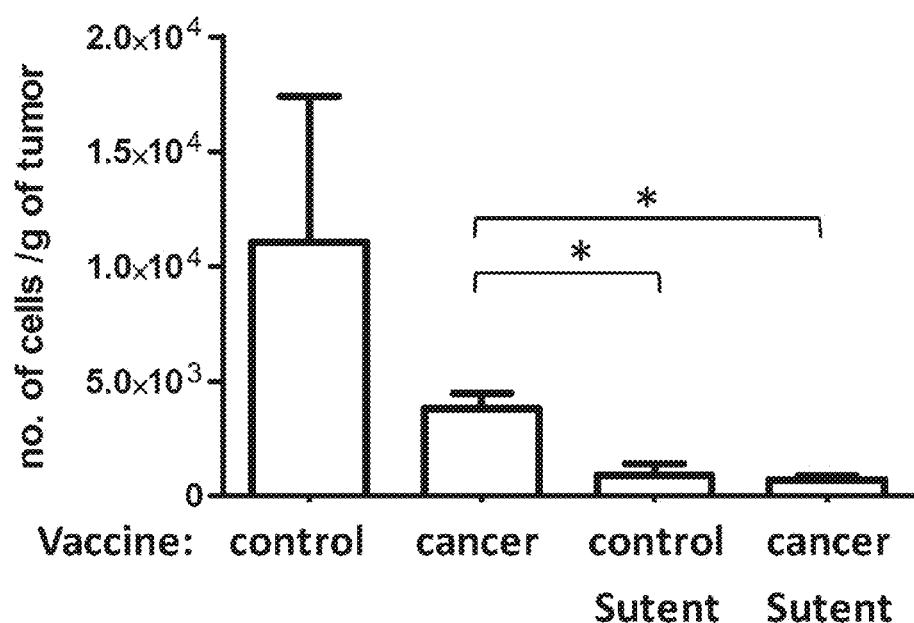
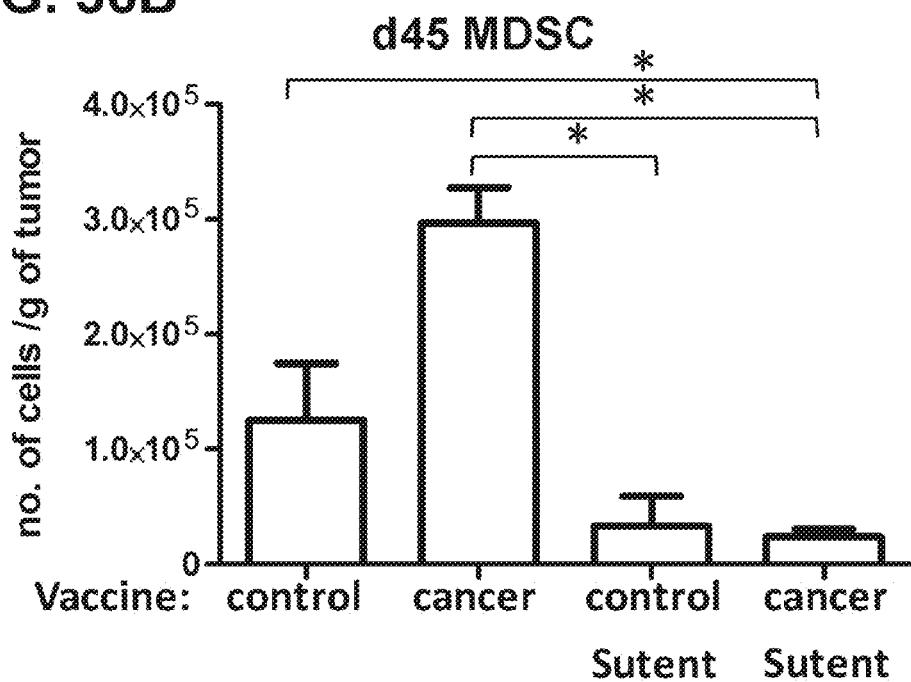
FIG. 36A**Treg****FIG. 36B****d45 MDSC**

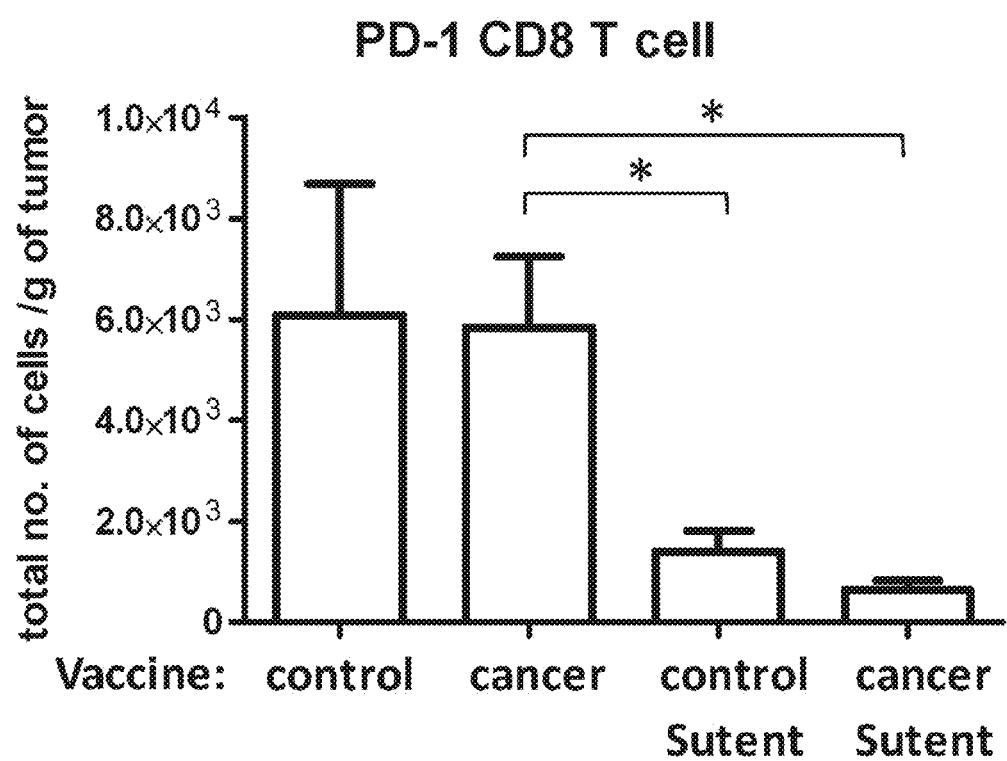
FIG. 36C

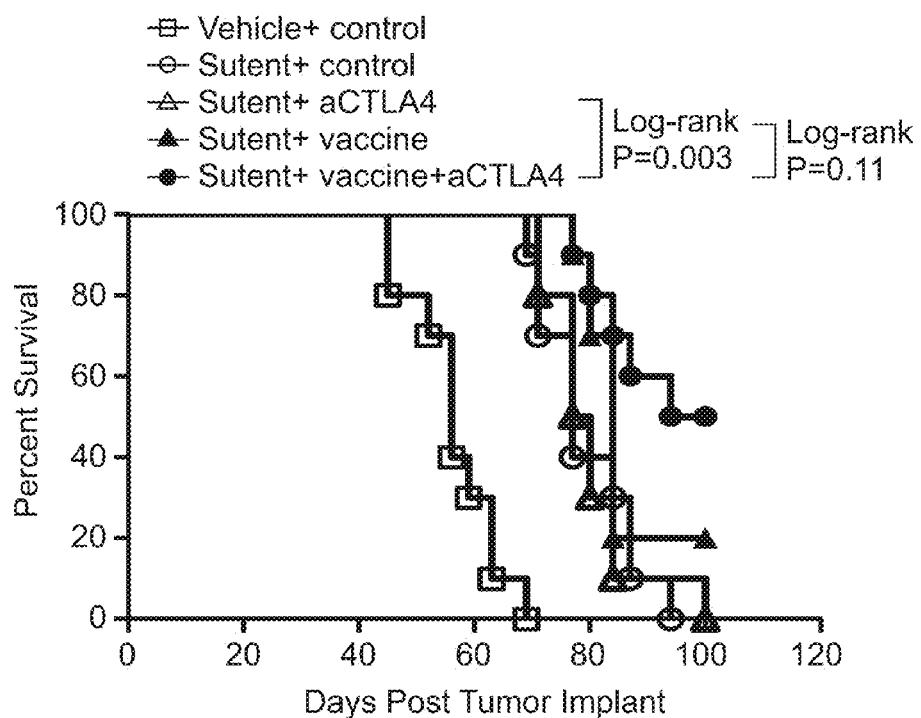
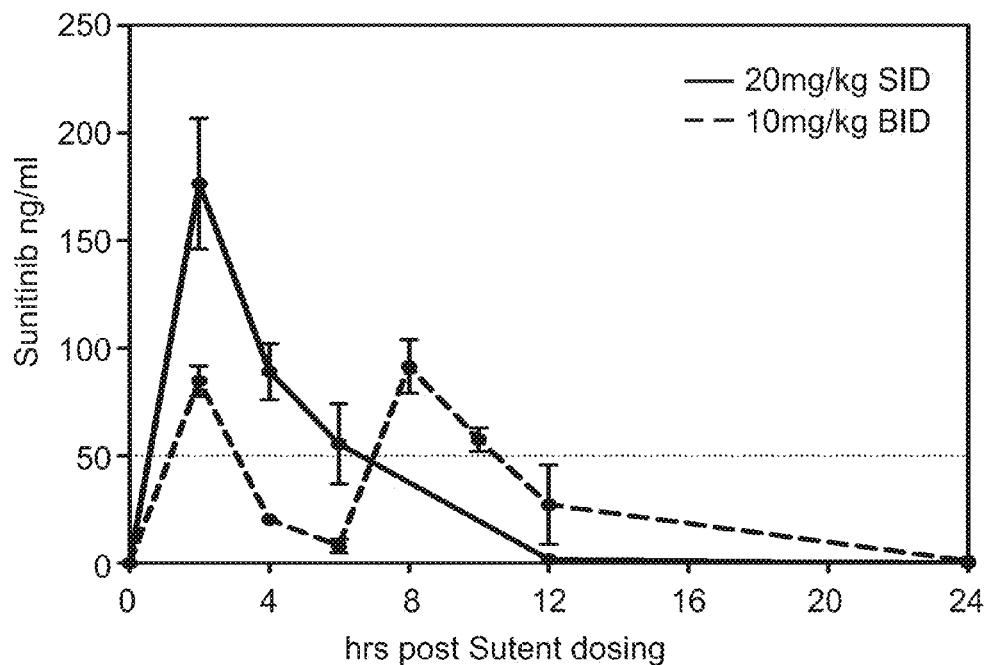
FIG. 37**FIG. 38**

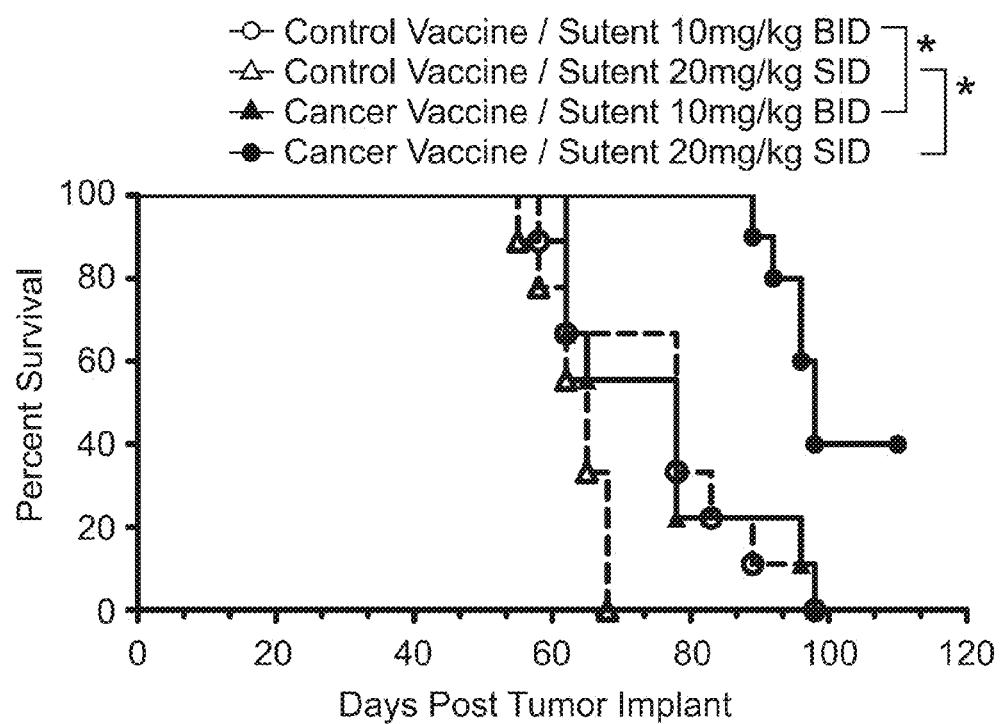
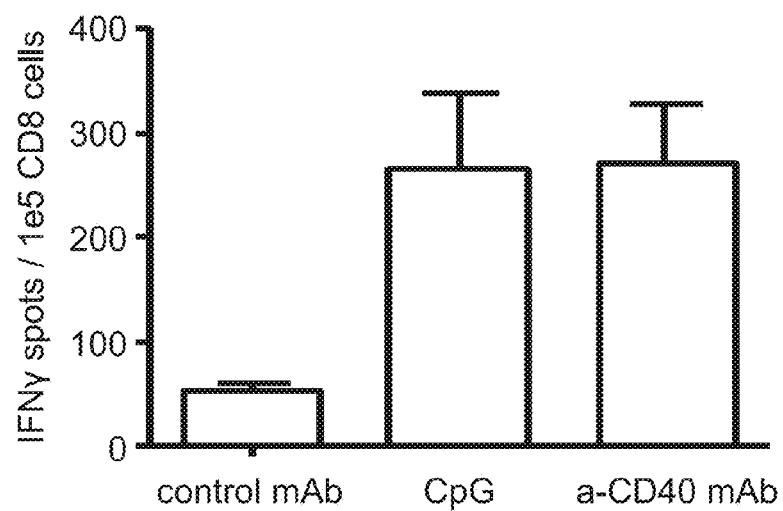
FIG. 39**FIG. 40**

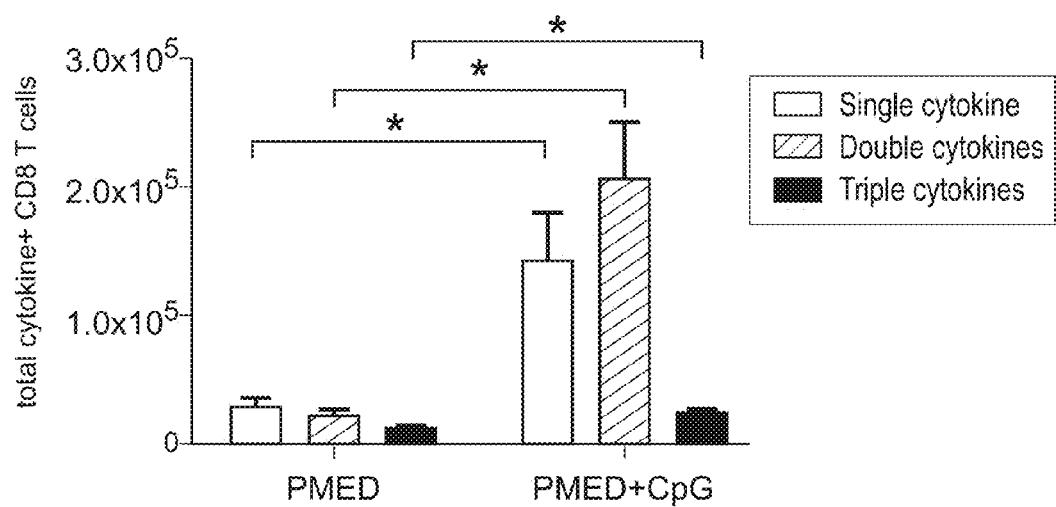
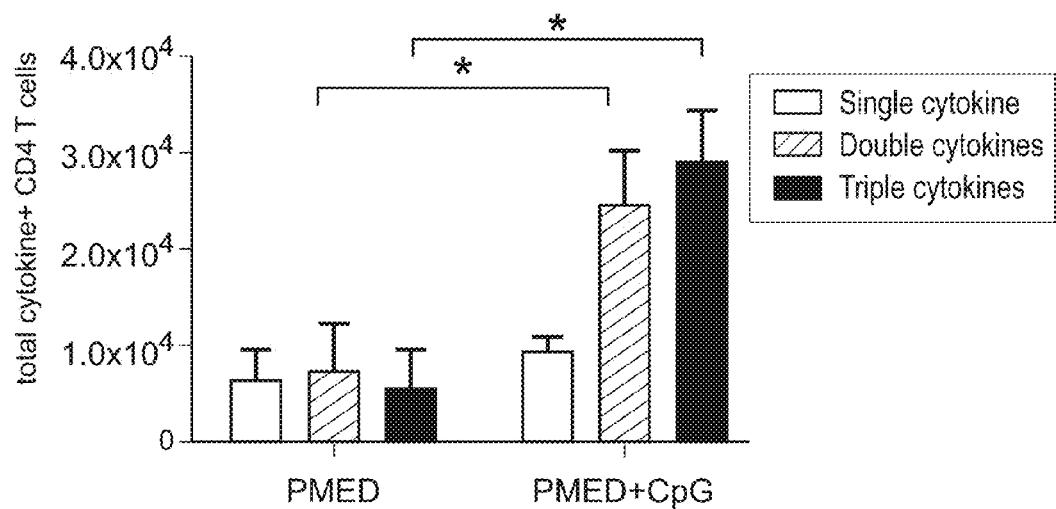
FIG. 41A**FIG. 41B**

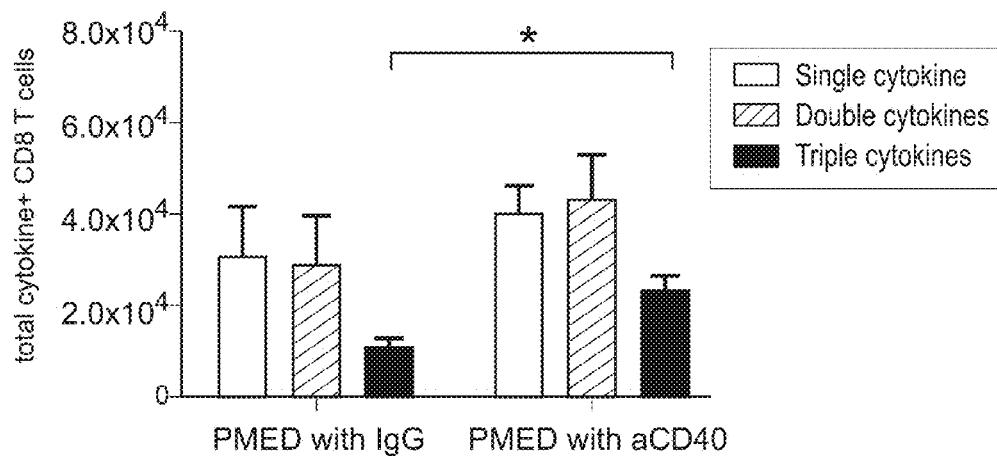
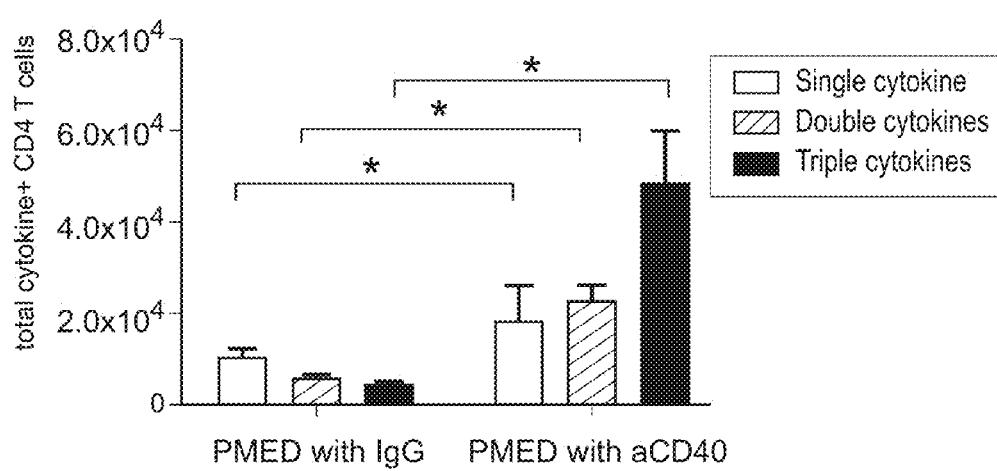
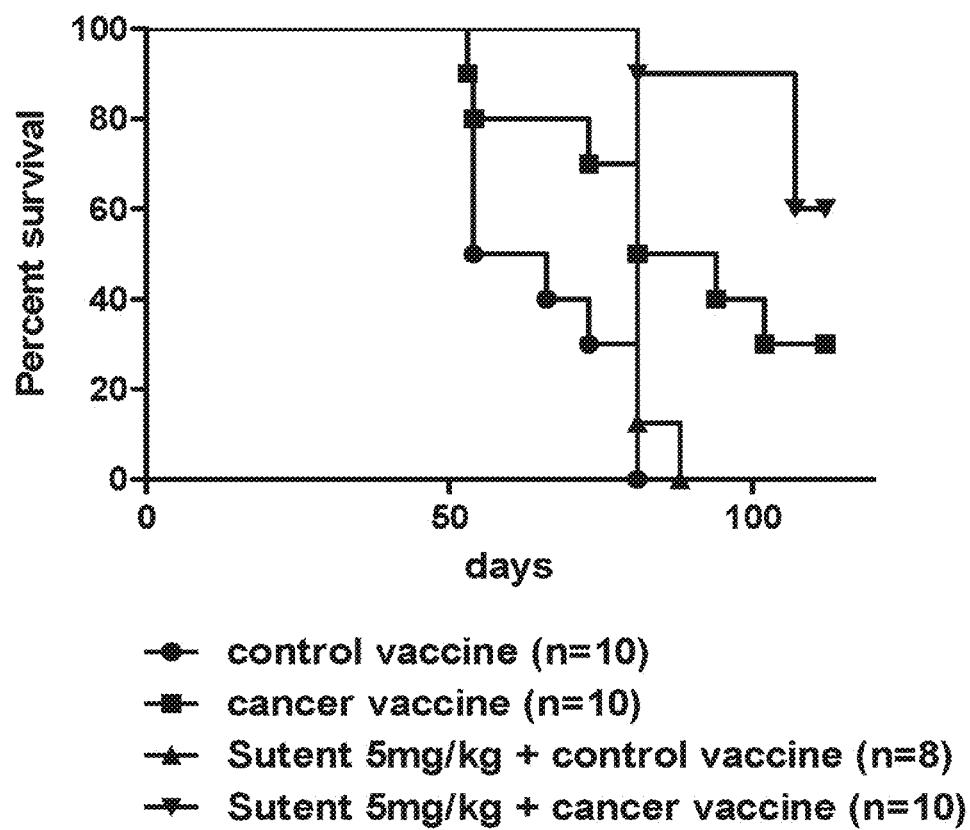
FIG. 42A**FIG. 42B**

FIG. 43

1

**PROSTATE-ASSOCIATED ANTIGENS AND
VACCINE-BASED IMMUNOTHERAPY
REGIMENS**

**CROSS-REFERENCE TO RELATED
APPLICATIONS**

This application is a division of application Ser. No. 13/875,162 filed on May 1, 2013, now allowed, which claims benefit of U.S. Provisional Application No. 61/642,844 filed on May 4, 2012. Both application Ser. No. 13/875,162 and U.S. Provisional Application No. 61/642,844 are incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING

This application is being filed along with a sequence listing in electronic format. The sequence listing is provided as a file in .txt format entitled "PC71854B_SEQ LISTING_ST25.TXT", created on Jan. 5, 2016 and having a size of 260 KB. The sequence listing contained in the .txt file is part of the specification and is herein incorporated by reference in its entity.

FIELD OF THE INVENTION

The present invention relates generally to immunotherapy and specifically to vaccines and methods for treating or preventing neoplastic disorders.

BACKGROUND OF THE INVENTION

Cancer is a leading cause of mortality worldwide. Traditional regimens of cancer management have been successful in the management of a selective group of circulating and solid cancers. However, many tumors are resistant to traditional approaches. In recent years, immunotherapy for the treatment of cancers has been explored, which involves the generation of an active systemic tumor-specific immune response of host origin by administering a vaccine composition at a site distant from the tumor. Various types of vaccines have been proposed, including those containing isolated tumor-associated antigens.

Prostate cancer is the second most commonly diagnosed cancer and the fourth leading cause of cancer-related death in men in the developed countries worldwide. Various prostate-associated antigens (PAA), such as prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA) have been shown to be overexpressed by prostate cancer cells as compared to normal counterparts. These antigens, therefore, represent possible targets for inducing specific immune responses against cancers expressing the antigens via the use of vaccine-based immunotherapy. (see e.g. Marrari, A., M. Iero, et al. (2007). "Vaccination therapy in prostate cancer." *Cancer Immunol Immunother* 56(4): 429-45.)

PSCA is a 123-amino acid membrane protein. The amino acid sequence of the full length human PSCA consists of amino acids 4-123 of SEQ ID NO:21. PSCA has high tissue specificity and is expressed on more than 85% of prostate cancer specimens, with expression levels increasing with higher Gleason scores and androgen independence. It is expressed in 80-100% of bone metastasis of prostate cancer patients.

PSA is a kallikrein-like serine protease that is produced exclusively by the columnar epithelial cells lining the acini and ducts of the prostate gland. PSA mRNA is translated as

2

an inactive 261-amino acid preproPSA precursor. Prepro-PSA has 24 additional residues that constitute the pre-region (the signal polypeptide) and the propolypeptide. Release of the propolypeptide results in the 237-amino acid, mature extracellular form, which is enzymatically active. The amino acid sequence of the human full length PSA is provided in SEQ ID NO: 15. PSA is organ-specific and, as a result, it is produced by the epithelial cells of benign prostatic hyperplastic (BPH) tissue, primary prostate cancer tissue, and metastatic prostate cancer tissue.

PSMA, also known as Folate hydrolase 1 (FOLH1), is composed of 750 amino acids. The amino acid sequence of the human full length PSMA is provided in SEQ ID NO:1. PSMA includes a cytoplasmic domain (amino acids 1-19), a transmembrane domain (amino acids 20-43), and an extracellular domain (amino acids 44-750). PSMA is a type II dimeric transmembrane protein expressed on the surface of prostate cancer cells and on neovasculature. It is also expressed on normal prostate cells, brain, salivary gland and biliary tree. However, in prostate cancer cells it was found to be expressed at 1000-fold higher levels than normal tissues. It is abundantly expressed on neovasculature of a variety of other solid tumors such as colon, breast, liver, bladder, pancreas, lung, renal cancers as well as melanoma and sarcomas. Thus, PSMA is considered a target not only specific for prostate cancer cells but also a pan-carcinoma target for other cancers. The expression of PSMA appears to be a universal feature of prostate carcinomas and its increased expression correlates with tumor aggressiveness. PSMA expression is highest in high-grade tumors, metastatic lesions and androgen-independent disease.

While a large number of tumor-associated antigens have been identified and many of these antigens have been explored as protein-based or DNA-based vaccines for the treatment or prevention of cancers, most clinical trials so far have failed to produce a therapeutic product. One of the challenges in developing cancer vaccines resides in the fact that the cancer antigens are usually self-derived and, therefore, poorly immunogenic because the immune system is self-regulated not to recognize self-proteins. Accordingly, a need exists for a method to enhance the immunogenicity or therapeutic effect of cancer vaccines.

Numerous approaches have been explored for enhancing the immunogenicity or enhancing anti-tumor efficacy of cancer vaccines. One of such approach involves the use of various immune modulators, such as TLR agonists, TNFR agonists, CTLA-4 inhibitors, and protein kinase inhibitors.

Toll-like receptors (TLRs) are type 1 membrane receptors that are expressed on hematopoietic and non-hematopoietic cells. At least 11 members have been identified in the TLR family. These receptors are characterized by their capacity to recognize pathogen-associated molecular patterns (PAMP) expressed by pathogenic organisms. It has been found that triggering of TLR elicits profound inflammatory responses through enhanced cytokine production, chemokine receptor expression (CCR2, CCR5 and CCR7), and co-stimulatory molecule expression. As such, these receptors in the innate immune systems exert control over the polarity of the ensuing acquired immune response. Among the TLRs, TLR9 has been extensively investigated for its functions in immune responses. Stimulation of the TLR9 receptor directs antigen-presenting cells (APCs) towards priming potent, T_{H1} -dominated T-cell responses, by increasing the production of pro-inflammatory cytokines and the presentation of co-stimulatory molecules to T cells. CpG oligonucleotides, ligands for TLR9, were found to be a class of potent immunostimulatory factors. CpG therapy has been tested

against a wide variety of tumor models in mice, and has consistently been shown to promote tumor inhibition or regression.

Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) is a member of the immunoglobulin superfamily and is expressed on the surface of Helper T cells. CTLA-4 is a negative regulator of CD28 dependent T cell activation, and acts as an inhibitory checkpoint for the adaptive immune response. Similar to the T-cell costimulatory protein CD28, CTLA-4 binds to CD80 and CD86 on antigen-presenting cells. CTLA-4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Human antibodies against human CTLA-4 have been described as immunostimulation modulators in a number of disease conditions, such as treating or preventing viral and bacterial infection and for treating cancer (WO 01/14424 and WO 00/37504). Various preclinical studies have shown that CTLA-4 blockade by monoclonal antibodies enhances the host immune response against immunogenic tumors, and can even reject established tumors. Two fully human anti-human CTLA-4 monoclonal antibodies (mAbs), ipilimumab (MDX-010) and Tremelimumab (also known as CP-675206), have been investigated in clinical trials in the treatment of various types of solid tumors.

The tumor necrosis factor (TNF) superfamily is a group of cytokines that engage specific cognate cell surface receptors, the TNF receptor (TNFR) superfamily. Members of the tumor necrosis factor superfamily act through ligand-mediated trimerization, causing recruitment of several intracellular adaptors to activate multiple signal transduction pathways, such as apoptosis, NF- κ B pathway, JNK pathway, as well as immune and inflammatory responses. Examples of the TNF Superfamily include CD40 ligands, OX40 ligands, 4-1BB ligands, CD27, CD30 ligand (CD153), TNF-alpha, TNF-beta, RANK ligands, LT-alpha, LT-beta, GITR ligands, and LIGHT. The TNFR Superfamily includes, for example, CD40, OX40, 4-1BB, CD70 (CD27 ligand), CD30, TNFR2, RANK, LT-beta R, HVEM, GITR, TROY, and RELT. CD40 is found on the surface of B lymphocytes, dendritic cells, follicular dendritic cells, hematopoietic progenitor cells, epithelial cells, and carcinomas. CD40 binds to a ligand (CD40-L), which is a glycoprotein and expressed on activated T cells, mostly CD4+ but also some CD8+ as well as basophils/mast cells. Because of the role of CD40 in innate and adaptive immune responses, CD40 agonists, including various CD40 agonistic antibodies, such as the fully human agonist CD40 monoclonal antibody CP870893, have been explored for usage as vaccine adjuvants and in therapies.

Protein kinases are a family of enzymes that catalyze the phosphorylation of specific residues in proteins. Protein kinases are key elements in signal transduction pathways responsible for transducing extracellular signals, including the action of cytokines on their receptors, to the nuclei, triggering various biological events. The many roles of protein kinases in normal cell physiology include cell cycle control and cell growth, differentiation, apoptosis, cell mobility and mitogenesis. Kinases such as c-Src, c-Abl, mitogen activated protein (MAP) kinase, phosphotidylinositol-3-kinase (PI3K) AKT, and the epidermal growth factor (EGF) receptor are commonly activated in cancer cells, and are known to contribute to tumorigenesis. Logically, a number of kinase inhibitors are currently being developed for anti-cancer therapy, in particular tyrosine kinase inhibitors (TKIs): cyclin-dependent kinase inhibitors, aurora kinase inhibitors, cell cycle checkpoint inhibitors, epidermal growth factor receptor (EGFR) inhibitors, FMS-like tyrosine kinase inhibitors, platelet-derived growth factor receptor

(PDGFR) inhibitors, kinase insert domain inhibitors, inhibitors targeting the PI3K/Akt/mTOR pathway, inhibitors targeting the Ras-Raf-MEK-ERK (ERK) pathway, vascular endothelial growth factor receptor (VEGFR) kinase inhibitors, c-kit inhibitors and serine/threonine kinase inhibitors. A number of kinase inhibitors have been investigated in clinical investigation for use in anti-cancer therapies, which includes, for example, MK0457, VX-680, ZD6474, MLN8054, AZD2171, SNS-032, PTK787/ZK222584, Sorafenib (BAY43-9006), SU5416, SU6668 AMG706, Zactima (ZD6474), MP-412, Dasatinib, CEP-701, (Lestaurtinib), XL647, XL999, Tykerb, (Lapatinib), MLN518, (formerly known as CT53518), PKC412, ST1571, AMN107, AEE 788, OSI-930, OSI-817, Sunitinib malate (Sutent; SU11248), Vatalanib (PTK787/ZK 222584), SNS-032, SNS-314 and Axitinib (AG-013736). Gefitinib and Erlotinib are two orally available EGFR-TKIs.

The immune modulators that have been explored are typically administered systemically to the patients, for example, by oral administration, intravenous injection or infusion, or intramuscular injection. One major factor that limits the effective use of some of the immune modulators is toxicity caused by high systemic exposure to the administered agents. For example, with respect to CD40 agonists, it has been reported that 0.3 mg/kg is the maximum tolerated dose for an exemplified agonistic CD40 antibody and that higher doses may elicit side effects including venous thromboembolism, grade 3 headache, cytokine release resulting in toxic effects such as chills and the like, and transient liver toxicity. (Vanderheide et al., *J Clin. Oncol.* 25(7): 876-8833 (March 2007)). In a clinical trial to investigate combinations of intravenous Tremelimumab (an anti-CTLA-4 antibody) plus oral sunitinib in patients with metastatic renal cell carcinoma, rapid onset of renal failure was observed and, as a result, further investigation of Tremelimumab at doses higher than 6 mg/kg plus sunitinib at 37.5 mg daily was not recommended. See: Brian I. Rini et al.: Phase 1 Dose-Escalation Trial of Tremelimumab Plus Sunitinib in Patients With Metastatic Renal Cell Carcinoma. *Cancer* 117(4):158-767 (2011)]. Therefore, there is a need for vaccine-based immunotherapy regimens where the immune modulators are administered at effective doses which do not elicit severe adverse side effects such as liver toxicity or renal failure.

SUMMARY OF THE INVENTION

In some aspects, the present disclosure provides isolated immunogenic PSMA polypeptides and immunogenic PSA polypeptides, which are useful, for example, for eliciting an immune response *in vivo* (e.g. in an animal, including humans) or *in vitro*, generating antibodies, or for use as a component in vaccines for treating cancers, including prostate cancer. In one aspect, the present disclosure provides isolated immunogenic PSMA polypeptides which have at least 90% identity to amino acids 15-750 of the human PSMA of SEQ ID NO:1 and comprise the amino acids of at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 of the conserved T cell epitopes of the human PSMA at corresponding positions.

In other aspects, the present disclosure provides nucleic acid molecules that encode immunogenic PAA polypeptides. In some embodiments, the present disclosure provides isolated nucleic acid molecules, or degenerate variants thereof, which comprise a nucleotide sequence encoding an immunogenic PSMA polypeptide, or a functional variant of said polypeptide, provided by the present disclosure.

5

In some other aspects, the present disclosure provides multi-antigen nucleic acid constructs that each encode two or more immunogenic PAA polypeptides.

The disclosure also provides vectors containing one or more nucleic acid molecules of the invention. The vectors are useful for cloning or expressing the immunogenic PAA polypeptides encoded by the nucleic acid molecules, or for delivering the nucleic acid molecules in a composition, such as a vaccine, to a host cell or to a host animal, such as a human.

In some further aspects, the present disclosure provides compositions comprising one or more immunogenic PAA polypeptides, isolated nucleic acid molecules encoding immunogenic PAA polypeptides, or vectors or plasmids containing nucleic acid molecules encoding immunogenic PAA polypeptides. In some embodiments, the composition is an immunogenic composition useful for eliciting an immune response against a PAA in a mammal, such as a mouse, dog, monkey, or human. In some embodiments, the composition is a vaccine composition useful for immunization of a mammal, such as a human, for inhibiting abnormal cell proliferation, for providing protection against the development of cancer (used as a prophylactic), or for treatment of disorders (used as a therapeutic) associated with PAA over-expression, such as cancer, particularly prostate cancer.

In still other aspects, the present disclosure provides methods of using the immunogenic PAA polypeptides, isolated nucleic acid molecules, and compositions comprising an immunogenic PAA polypeptide or isolated nucleic acid molecules described herein above. In some embodiments, the present disclosure provides a method of eliciting an immune response against a PAA in a mammal, particularly a human, comprising administering to the mammal an effective amount of a polypeptide provided by the invention that is immunogenic against the target PAA, an effective amount of an isolated nucleic acid molecule encoding such an immunogenic polypeptide, or a composition comprising such an immunogenic PAA polypeptide or an isolated nucleic acid molecule encoding such an immunogenic PAA polypeptide. The polypeptide or nucleic acid vaccines may be used together with one or more adjuvants.

In yet other aspects, the present disclosure provides vaccine-based immunotherapy regimens (or “VBIR”) that involve co-administration of a vaccine delivering various tumor associated antigens (TAAs) for inducing TAA specific immune responses to treat a variety of cancers in combination with at least one immune-suppressive-cell inhibitor and at least one immune-effector-cell enhancer. Specifically, in some aspects, the disclosure provides a method of enhancing the immunogenicity or therapeutic effect of a vaccine for the treatment of a neoplastic disorder in a mammal, comprising administering to the mammal receiving the vaccine an effective amount of at least one immune-suppressive-cell inhibitor and at least one immune-effector-cell enhancer. In a further aspect, the disclosure provides a method of treating a neoplastic disorder in a mammal, comprising administering to the mammal a vaccine, at least one immune-suppressive-cell inhibitor, and at least one immune-effector-cell enhancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Schematic illustration of PJV7563 vector.

FIG. 2. Amino acid alignment of five viral 2A cassettes, which are FMDV 2A (SEQ ID NO:57), TAV 2A (SEQ ID NO:58), EMCV 2A (SEQ ID NO:59), ERAV 2A (SEQ ID

6

NO:60), and PTV 2A (SEQ ID NO:61). The skipped glycine-proline bonds are indicated by asterisks.

FIG. 3. Sequence of the preferred EMCV IRES (SEQ ID NO:62). The translation initiation site is indicated by the asterisk. The minimal IRES element excludes the underlined first 5 codons of the EMCV L protein.

FIG. 4. Dot plots showing expression of the human PSMA modified antigen (amino acids 15-750) and full length human PSCA on the surface of HEK293 cells transfected with dual antigen vaccine constructs as measured by flow cytometry.

FIGS. 5A and 5B. Image of Western blots showing expression of the human PSMA modified antigen (amino acids 15-750; FIG. 5A) and full length human PSCA (FIG. 5B) in HEK293 cells transfected with dual antigen vaccine constructs as measured by western blotting with PSMA and PSCA specific monoclonal antibodies.

FIG. 6. Image of Western blots showing expression of human PSA cytosolic antigen (amino acids 25-261) in HEK293 cells transfected with dual antigen vaccine constructs as measured by western blotting with a PSA specific monoclonal antibody. Lane 5300 exhibited a faint band about 2 kD larger than PSA, consistent with a C-terminal fusion of the 2A peptide.

FIGS. 7A, 7B. Dot plots showing expression of human PSMA modified antigen (amino acids 15-750) and full length human PSCA on the surface of HEK293 cells transfected with either single promoter triple antigen constructs (FIG. 7A) or dual promoter triple antigen vaccine constructs (FIG. 7B) as measured by flow cytometry.

FIGS. 8A, 8B. Images of Western blots showing expression of human PSA in HEK293 cells transfected with either single promoter triple antigen constructs (FIG. 8A) or dual promoter triple antigen vaccine constructs (FIG. 8B) as measured by western blotting with a PSA specific monoclonal antibody. The bands in lanes 5259 and 456 are spillover from lane 5297. Although not visible in the scanned gel, lanes 456, 457, and 458 exhibited a band about 2 kD larger than PSA.

FIGS. 9A-9D. Graphs depicting results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by IFN- γ ELISPOT assay, in which recognition of endogenous prostate antigens was assessed by examining T cell responses to (a) TRAMP C2 cells expressing PSMA (FIG. 9A), (b) TRAMP C2 cells expressing PSCA (FIG. 9B), (c) TRAMP C2 cells expressing PSA (FIG. 9C), and (d) TRAMP C2 cells expressing PSMA, PSA, and PSCA (FIG. 9D).

FIGS. 10A-10D. Graphs depicting results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by IFN- γ ELISPOT assay, in which T cell responses to (a) individual PSMA peptides (FIG. 10A), (b) three PSMA peptide pools (FIG. 10B), (c) a PSCA peptide (FIG. 10C), and (d) PSA peptides (FIG. 10D) were assessed.

FIG. 11. Graph depicting results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSMA antibody titers.

FIG. 12. Graph depicting results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSCA antibody titers.

FIG. 13. Graph depicting results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSMA antibody cell-surface binding.

FIG. 14. Graph depicting results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSCA antibody cell-surface binding.

FIGS. 15A-15C. Graphs depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by IFN- γ ELISPOT assay, in which recognition of endogenous prostate antigens was assessed by examining T cell responses to (a) TRAMP C2 cells expressing PSMA (FIG. 15A), (b) TRAMP C2 cells expressing PSCA (FIG. 15B), and (c) TRAMP C2 cells expressing PSA, PSA, and PSCA (FIG. 15C).

FIGS. 16A-16C. Graphs depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by IFN- γ ELISPOT assay, in which T cell responses to (a) individual PSMA peptides (FIG. 16A), (b) three different PSMA peptide pools (FIG. 16B), and (c) a PSCA peptide (FIG. 16C) were assessed.

FIG. 17. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody titers.

FIG. 18. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody titers.

FIG. 19. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody cell-surface binding.

FIG. 20. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody cell-surface binding.

FIGS. 21A-21D. Graphs depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by IFN- γ ELISPOT assay, in which recognition of endogenous PSMA, PSCA, and PSA was assessed by examining T cell responses to (a) TRAMP C2 cells expressing PSMA (FIG. 21A), (b) TRAMP C2 cells expressing PSCA (FIG. 21B), (c) TRAMP C2 cells expressing PSA (FIG. 21C), and (d) TRAMP C2 cells expressing PSMA, PSA, and PSCA (FIG. 21D).

FIG. 22. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody titers.

FIG. 23. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody titers.

FIG. 24. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody cell-surface binding.

FIG. 25. Graph depicting results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody cell-surface binding.

FIG. 26. Graph depicting results of a representative study that evaluates the T cell immune response elicited by human PSMA modified antigen (amino acids 15-750) versus full-length human PSMA (amino acids 1-750) in C57BL/6 mice.

FIGS. 27A, 27B. Graphs depicting results of a representative study that evaluates the T cell immune response of human PSMA modified antigen (amino acids 15-750) versus full-length human PSMA antigen (amino acids 1-750) in Pasteur (HLA-A2/DR1) transgenic mice by IFN- γ ELISPOT assay using (a) PSMA derived HLA-A2-restricted peptides (FIG. 27A) or (b) SK-Mel5 cells transduced with Ad-hPSMA or purified hPSMA full-length protein (FIG. 27B).

FIG. 28. Graph depicting results of a representative study that evaluates the immunogenicity of the human modified and full-length PSMA vaccines by anti-PSMA antibody titers.

FIG. 29. Graph depicting results of a representative study that evaluates the immunogenicity of the human modified and full-length PSMA vaccines by anti-PSMA antibody cell-surface binding.

FIG. 30. Graph depicting results of a representative study that evaluates the blood anti-CTLA-4 monoclonal antibody levels measured by competitive ELISA in Indian Rhesus macaques injected with anti-CTLA-4 (CP-675, 206) at 10 mg/kg.

FIGS. 31A and 31B. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of anti-murine CTLA-4 monoclonal antibody (clone 9H10) on the quality of the immune responses induced by a rat Her-2 DNA vaccine using an intracellular cytokine staining assay, in which (a) cytokine positive CD8 T cells (FIG. 31A) and (b) cytokine positive CD4 T cells (FIG. 31B) were measured.

FIG. 32. Graph depicting results of a representative study that evaluates the effect of sunitinib malate (Sutent) on the anti-tumor efficacy of a cancer vaccine (rHER2) in mice, in which the subcutaneous tumor growth rate was measured.

FIGS. 33A-33D. Graphs depicting results from a representative study that evaluates the effect of sunitinib malate (Sutent) on the anti-tumor efficacy of a cancer vaccine (rHER2), in which individual tumor growth rates were measured in mice treated with (a) the control agents (FIG. 33A), (b) sunitinib malate and the control vaccine (FIG. 33B), (c) the vehicle and the cancer vaccine (FIG. 33C), or (d) sunitinib malate and the cancer vaccine (FIG. 33D).

FIG. 34. Graph showing the Kaplan-Meier survival curves of the groups of mice from the study described in FIGS. 33A-33D that evaluates the effect of sunitinib malate (Sutent) on the anti-tumor efficacy of a cancer vaccine (rHER2).

FIGS. 35A, 35B. Graphs showing changes in myeloid derived suppressor cells (Gr1+CD11b+) (FIG. 35A) and Treg containing CD25+CD4+ cells (FIG. 35B) in the periphery blood of the groups of mice from the study described FIGS. 33A-33D.

FIGS. 36A-36C. Graphs depicting results of a representative study in a mouse tumor model that evaluates the effect of sunitinib malate (Sutent) on the total number of (a) Tregs (CD4+CD25+Foxp3+) (FIG. 36A), (b) myeloid derived suppressor cells (Gr1+CD11b+) (FIG. 36B), and (c) PD-1+CD8 T cells (FIG. 36C) isolated from tumors of the mice.

FIG. 37. Graph showing the Kaplan-Meier survival curves of the groups of mice from a representative study evaluating the effect of sunitinib malate (Sutent) and an anti-murine CTLA-4 monoclonal antibody (clone 9D9) on the anti-tumor efficacy of a cancer vaccine (vaccine) in subcutaneous TUBO tumor bearing BALB/neuT mice.

FIG. 38. Graph showing kinetics of the blood sunitinib levels of BALB/neuT mice with subcutaneous TUBO tumors.

FIG. 39. Graph showing the Kaplan-Meier survival curves of the groups of mice from a representative study that evaluates the effect of sunitinib malate (Sutent) on the anti-tumor efficacy of a cancer vaccine in BALB/neuT mice with subcutaneous TUBO tumors.

FIG. 40. Graph depicting the IFN γ ELISPOT results from a representative study evaluating the effect of CpG7909 and an anti-CD40 antibody (Bioxcell #BE0016-2) on the antigen specific T cell responses induced by a cancer vaccine (rHER2).

FIGS. 41A, 41B. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of CpG7909 on the quality of the immune responses induced by a cancer vaccine (PMED) using intracellular cytokine staining assay, in which cytokine positive CD8 T cells (FIG. 41A) and cytokine positive CD4 T cells (FIG. 41B) were measured.

FIGS. 42A, 42B. Graphs depicting results of a representative study that evaluates the immunomodulatory activity of an agonistic anti-murine CD40 monoclonal antibody on the quality of the immune responses induced by a cancer vaccine (PMED) using intracellular cytokine staining assay, in which cytokine positive CD8 T cells (FIG. 42A) and cytokine positive CD4 T cells (FIG. 42B) were measured.

FIG. 43. Graph showing the Kaplan-Meier survival curves of the groups of mice from a representative study that evaluates the effect of low dose sunitinib malate (Sutent) on the anti-tumor efficacy of a cancer vaccine in spontaneous mammary tumor bearing BALB/neuT mice.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

The term “adjuvant” refers to a substance that is capable of enhancing, accelerating, or prolonging an immune response when given with a vaccine immunogen.

The term “agonist” refers to a substance which promotes (induces, causes, enhances or increases) the activity of another molecule or a receptor. The term agonist encompasses substances which bind receptor (e.g., an antibody, a homolog of a natural ligand from another species) and substances which promote receptor function without binding thereto (e.g., by activating an associated protein).

The term “antagonist” or “inhibitor” refers to a substance that partially or fully blocks, inhibits, or neutralizes a biological activity of another molecule or receptor.

The term “co-administration” refers to administration of two or more agents to the same subject during a treatment period. The two or more agents may be encompassed in a single formulation and thus be administered simultaneously. Alternatively, the two or more agents may be in separate physical formulations and administered separately, either sequentially or simultaneously to the subject. The term “administered simultaneously” or “simultaneous administration” means that the administration of the first agent and that of a second agent overlap in time with each other, while the term “administered sequentially” or “sequential administration” means that the administration of the first agent and that of a second agent does not overlap in time with each other.

The term “conserved T cell epitope” refers to one of the following amino acid sequences of the human PSMA protein as set forth in SEQ ID NO. 1:

amino acids 168-176 (GMPEGDLVY),
 amino acids 347-356 (HSTNGVTRIY),
 amino acids 557-566 (ETYELVEKFY),
 amino acids 207-215 (KVFRGNKVK),
 amino acids 431-440 (STEWAEEENSR),
 amino acids 4-12 (LLHETDSAV),
 amino acids 27-35 (VLAGGFFLL),
 amino acids 168-177 (GMPEGDLVYV),
 amino acids 441-450 (LLQERGVAYI),
 amino acids 469-477 (LMYSLVHNL),
 amino acids 711-719 (ALFDIESKV),
 amino acids 663-671 (MNDQVMFL),
 amino acids 178-186 (NYARTEDFF),
 amino acids, 227-235 (LYSDPADYF),
 amino acids 624-632 (TYSVSFDSL),
 amino acids 334-348 (TGNFSTQKVKMHIHS),
 amino acids 459-473 (NYTLRVDCTPLMYSL),
 amino acids 687-701(YRHVIYAPSSHNKYA), and
 amino acids 730-744 (RQIYVAAFTVQAAAE).

The term “cytosolic” means that after a nucleotide sequence encoding a particular polypeptide is expressed by a host cell, the expressed polypeptide is retained inside the host cell.

5 The terms “degenerate variant” refers to DNA sequences that have substitutions of bases but encode the same polypeptide.

The term “effective amount” refers to an amount administered to a mammal that is sufficient to cause a desired effect in the mammal.

The term “fragment” of a given polypeptide refers to a polypeptide that is shorter than the given polypeptide and shares 100% identity with the sequence of the given polypeptide.

15 The term “identical” or percent “identity,” in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence.

The term “immune-effector-cell enhancer” or “IEC enhancer” refers to a substance capable of increasing or enhancing the number, quality, or function of one or more types of immune effector cells of a mammal. Examples of immune effector cells include cytolytic CD8 T cells, CD40 T cells, NK cells, and B cells.

20 The term “immune modulator” refers to a substance capable of altering (e.g., inhibiting, decreasing, increasing, enhancing or stimulating) the working of any component of the innate, humoral or cellular immune system of a mammal. Thus, the term “immune modulator” encompasses the “immune-effector-cell enhancer” as defined herein and the “immune-suppressive-cell inhibitor” as defined herein, as well as substance that affects other components of the immune system of a mammal.

The term “immune response” refers to any detectable response to a particular substance (such as an antigen or immunogen) by the immune system of a host vertebrate animal, including, but not limited to, innate immune responses (e.g., activation of Toll receptor signaling cascade), cell-mediated immune responses (e.g., responses mediated by T cells, such as antigen-specific T cells, and non-specific cells of the immune system), and humoral immune responses (e.g., responses mediated by B cells, such as generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids). Examples of immune responses include an alteration (e.g., increase) in Toll-like receptor activation, lymphokine (e.g., cytokine (e.g., Th1, Th2 or

30 Th17 type cytokines) or chemokine) expression or secretion, macrophage activation, dendritic cell activation, T cell (e.g., CD4+ or CD8+ T cell) activation, NK cell activation, B cell activation (e.g., antibody generation and/or secretion), binding of an immunogen (e.g., antigen (e.g., immunogenic polypeptide)) to an MHC molecule, induction of a

35 cytotoxic T lymphocyte (“CTL”) response, induction of a B cell response (e.g., antibody production), and, expansion (e.g., growth of a population of cells) of cells of the immune system (e.g., T cells and B cells), and increased processing and presentation of antigen by antigen presenting cells. The term “immune response” also encompasses any detectable response to a particular substance (such as an antigen or immunogen) by one or more components of the immune system of a vertebrate animal in vitro.

40 45 50 55 60 65 70 75 80 85 90 95 The term “immunogenic” refers to the ability of a substance to cause, elicit, stimulate, or induce an immune response, or to improve, enhance, increase or prolong a

11

pre-existing immune response, against a particular antigen, whether alone or when linked to a carrier, in the presence or absence of an adjuvant.

The term "immunogenic PSA polypeptide" refers to a polypeptide that is immunogenic against human PSA protein or against cells expressing human PSA protein.

The term "immunogenic PSCA polypeptide" refers to a polypeptide that is immunogenic against human PSCA protein or against cells expressing human PSCA protein.

The term "immunogenic PSMA polypeptide" refers to a polypeptide that is immunogenic against human PSMA protein or against cells expressing human PSMA protein.

The term "immunogenic PAA polypeptide" refers to an "immunogenic PSA polypeptide," an "immunogenic PSCA polypeptide," or an "immunogenic PSMA polypeptide" as defined herein above.

The term "immunogenic PSA nucleic acid molecule" refers to a nucleic acid molecule that encodes an immunogenic PSA polypeptide as defined herein.

The term "immunogenic PSCA nucleic acid molecule" refers to a nucleic acid molecule that encodes an "immunogenic PSCA polypeptide" as defined herein.

The term "immunogenic PSMA nucleic acid molecule" refers to a nucleic acid molecule that encodes an "immunogenic PSMA polypeptide" as defined herein.

The term "immunogenic PAA nucleic acid molecule" refers to a nucleic acid molecule that encodes an "immunogenic PSA polypeptide," an "immunogenic PSCA polypeptide," or an "immunogenic PSMA polypeptide" as defined herein above.

The term "immune-suppressive-cell inhibitor" or "ISC inhibitor" refers to a substance capable of reducing or suppressing the number or function of immune suppressive cells of a mammal. Examples of immune suppressive cells include regulatory T cells ("T regs"), myeloid-derived suppressor cells, and tumor-associated macrophages.

The term "intradermal administration," or "administered intradermally," in the context of administering a substance, such as a therapeutic agent or an immune modulator, to a mammal including a human, refers to the delivery of the substance into the dermis layer of the skin of the mammal. The skin of a mammal is composed of three layers—the epidermis, dermis, and subcutaneous layer. The epidermis is the relatively thin, tough, outer layer of the skin. Most of the cells in the epidermis are keratinocytes. The dermis, the skin's next layer, is a thick layer of fibrous and elastic tissue (made mostly of collagen, elastin, and fibrillin) that gives the skin its flexibility and strength. The dermis contains nerve endings, sweat glands and oil (sebaceous) glands, hair follicles, and blood vessels. The dermis varies in thickness depending on the location of the skin. In humans it is about 0.3 mm on the eyelid and about 3.0 mm on the back. The subcutaneous layer is made up of fat and connective tissue that houses larger blood vessels and nerves. The thickness of this layer varies throughout the body and from person to person. The term "intradermal administration" refers to delivery of a substance to the inside of the dermis layer. In contrast; "subcutaneous administration" refers to the administration of a substance into the subcutaneous layer and "topical administration" refers to the administration of a substance onto the surface of the skin.

The term "local administration" or "administered locally" encompasses "topical administration," "intradermal administration," and "subcutaneous administration," each as defined herein above. This term also encompasses "intratumoral administration," which refers to administration of a substance to the inside of a tumor. Local administration is

12

intended to allow for high local concentrations around the site of administration for a period of time until systemic biodistribution has been achieved with of the administered substance, while "systemic administration" is intended for the administered substance to be absorbed into the blood and attain systemic exposure rapidly by being distributed through the circulatory system to organs or tissues throughout the body.

The term "mammal" refers to any animal species of the Mammalia class. Examples of mammals include: humans; non-human primates such as monkeys; laboratory animals such as rats, mice, guinea pigs; domestic animals such as cats, dogs, rabbits, cattle, sheep, goats, horses, and pigs; and captive wild animals such as lions, tigers, elephants, and the like.

The term "membrane-bound" means that after a nucleotide sequence encoding a particular polypeptide is expressed by a host cell, the expressed polypeptide is bound to, attached to, or otherwise associated with, the membrane of the cell.

The term "neoplastic disorder" refers to a condition in which cells proliferate at an abnormally high and uncontrolled rate, the rate exceeding and uncoordinated with that of the surrounding normal tissues. It usually results in a solid lesion or lump known as "tumor." This term encompasses benign and malignant neoplastic disorders. The term "malignant neoplastic disorder", which is used interchangeably with the term "cancer" in the present disclosure, refers to a neoplastic disorder characterized by the ability of the tumor cells to spread to other locations in the body (known as "metastasis"). The term "benign neoplastic disorder" refers to a neoplastic disorder in which the tumor cells lack the ability to metastasize.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a transgene is ligated in such a way that expression of the transgene is achieved under conditions compatible with the control sequences.

The term "ortholog" refers to genes in different species that are similar to each other and originated from a common ancestor.

The term "pharmaceutically acceptable excipient" refers to a substance in an immunogenic or vaccine composition, other than the active ingredients (e.g., the antigen, antigen-coding nucleic acid, immune modulator, or adjuvant) that is compatible with the active ingredients and does not cause significant untoward effect in subjects to whom it is administered.

The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically, or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones.

The term "preventing" or "prevent" refers to (a) keeping a disorder from occurring or (b) delaying the onset of a disorder or onset of symptoms of a disorder.

The term "prostate-associated-antigen" (or PAA) refers to the TAA (as defined herein) that is specifically expressed on prostate tumor cells or expressed at a higher frequency or density by tumor cells than by non-tumor cells of the same tissue type. Examples of PAA include PSA, PSCA, and PSMA.

The term "secreted" in the context of a polypeptide means that after a nucleotide sequence encoding the polypeptide is

13

expressed by a host cell, the expressed polypeptide is secreted outside of the host cell.

The term "suboptimal dose" when used to describe the amount of an immune modulator, such as a protein kinase inhibitor, refers to a dose of the immune modulator that is below the minimum amount required to produce the desired therapeutic effect for the disease being treated when the immune modulator is administered alone to a patient.

The term "treating," "treatment," or "treat" refers to abrogating a disorder, reducing the severity of a disorder, or reducing the severity or occurrence frequency of a symptom of a disorder.

The term "tumor-associated antigen" or "TAA" refers to an antigen which is specifically expressed by tumor cells or expressed at a higher frequency or density by tumor cells than by non-tumor cells of the same tissue type. Tumor-associated antigens may be antigens not normally expressed by the host; they may be mutated, truncated, misfolded, or otherwise abnormal manifestations of molecules normally expressed by the host; they may be identical to molecules normally expressed but expressed at abnormally high levels; or they may be expressed in a context or milieu that is abnormal. Tumor-associated antigens may be, for example, proteins or protein fragments, complex carbohydrates, gangliosides, haptens, nucleic acids, or any combination of these or other biological molecules.

The term "vaccine" refers to an immunogenic composition for administration to a mammal for eliciting an immune response against a particular antigen.

The term "variant" of a given polypeptide refers to a polypeptide that shares less than 100% but more than 80% identity to the amino acid sequence of that given polypeptide and exhibits at least some of the immunogenic activity of that given polypeptide.

The term "vector" refers to a nucleic acid molecule capable of transporting or transferring a foreign nucleic acid molecule. The term encompasses both expression vectors and transcription vectors. The term "expression vector" refers to a vector capable of expressing the insert in the target cell, and generally contain control sequences, such as enhancer, promoter, and terminator sequences, that drive expression of the insert. The term "transcription vector" refers to a vector capable of being transcribed but not translated. Transcription vectors are used to amplify their insert. The foreign nucleic acid molecule is referred to as "insert" or "transgene." A vector generally consists of an insert and a larger sequence that serves as the backbone of the vector. Based on the structure or origin of vectors, major types of vectors include plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as adenovirus (Ad) vectors, and artificial chromosomes.

B. Immunogenic Prostate-Associated-Antigen (PAA) Polypeptides

In some aspects, the present disclosure provides isolated immunogenic PSA polypeptides and PSMA polypeptides, which are useful, for example, for eliciting an immune response in vivo (e.g. in an animal, including humans) or in vitro, activating effector T cells, or generating antibodies specific for PSA and PSMA, respectively, or for use as a component in vaccines for treating cancer, particularly prostate cancer. These polypeptides can be prepared by methods known in the art in light of the present disclosure. The capability of the polypeptides to elicit an immune response can be measured in in vitro assays or in vivo assays. In vitro assays for determining the capability of a polypeptide or

14

DNA construct to elicit immune responses are known in the art. One example of such in vitro assays is to measure the capability of the polypeptide or nucleic acid expressing an polypeptide to stimulate T cell response as described in U.S. Pat. No. 7,387,882, the disclosure of which is incorporated in this application. The assay method comprises the steps of: (1) contacting antigen presenting cells in culture with an antigen thereby the antigen can be taken up and processed by the antigen presenting cells, producing one or more processed antigens; (2) contacting the antigen presenting cells with T cells under conditions sufficient for the T cells to respond to one or more of the processed antigens; (3) determining whether the T cells respond to one or more of the processed antigens. The T cells used may be CD8⁺ T cells or CD4⁺ T cells. T cell response may be determined by measuring the release of one or more of cytokines, such as interferon-gamma and interleukin-2, lysis of the antigen presenting cells (tumor cells), and production of antibodies by B cells.

B-1. Immunogenic PSMA Polypeptides

In one aspect, the present disclosure provides isolated immunogenic PSMA polypeptides which have at least 90% identity to amino acids 15-750 of the human PSMA of SEQ ID NO:1 and comprise the amino acids of at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 of the conserved T cell epitopes of the human PSMA at corresponding positions.

In some embodiments, the immunogenic PSMA polypeptides comprise at least 15, 16, 17, 18, or 19 of the conserved T cell epitopes of the human PSMA.

In some embodiments, the present disclosure provides an immunogenic PSMA polypeptide consisting of the amino acid sequence of SEQ ID NO:9, or an immunogenic PSMA polypeptide having 93%-99%, 94%-98%, or 94%-97% identity to the amino acid sequence of SEQ ID NO:9.

Examples of some particular immunogenic PSMA polypeptides include:

- 1) a polypeptide consisting of amino acids 15-750 of SEQ ID NO: 1;
- 2) a polypeptide comprising the amino acids 4-739 of SEQ ID NO: 3;
- 3) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:5;
- 4) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:7;
- 2) a polypeptide comprising the amino acid sequence of SEQ ID NO:3;
- 3) a polypeptide comprising the amino acid sequence of SEQ ID NO:5; and
- 4) a polypeptide comprising the amino acid sequence of SEQ ID NO:7.

In other embodiments, the present disclosure provides an immunogenic PSMA polypeptide selected from the group consisting of:

- 1) a polypeptide consisting of the amino acid sequence of SEQ ID NO:11
- 2) a polypeptide consisting of the amino acid sequence of SEQ ID NO:13; and
- 3) a polypeptide comprising the amino acid sequence of SEQ ID NO:13.

In some other embodiments, the present disclosure provides isolated immunogenic PSMA polypeptides that are variants of any of the following polypeptides:

- 2) a polypeptide comprising the amino acids 4-739 of SEQ ID NO: 3;
- 3) a polypeptide comprising the amino acids 4-739 of SEQ ID NO: 5; and

15

4) a polypeptide comprising the amino acids 4-739 of SEQ ID NO: 7, wherein the amino acid sequence of the variant has 93%-99% identity to the sequence of SEQ ID NO:1 and share at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity with the amino acid sequence of SEQ ID NO: 3, 5, or 7.

The variants of a given PAA polypeptide can be obtained by deleting, inserting, or substituting one or more amino acids in the parent immunogenic PAA polypeptide. An example for the production of such variants is the conservative substitution of individual amino acids of the polypeptides, that is, by substituting one amino acid for another having similar properties.

An immunogenic PSMA polypeptide of the invention may be constructed by conserving some or all of the conserved T cell epitopes of the human PSMA of SEQ ID NO:1 while substituting certain amino acids in the remaining regions of the human PSMA with amino acids found in one or more orthologs of human PSMA at corresponding positions. Sequences of various PSMA orthologs that may be utilized to make the immunogenic PSMA polypeptides are available from the GeneBank database. These orthologs along with their NCBI ID numbers are provided in Table 18. Substitutions of amino acids of human PSMA with amino acids from one or more of the orthologs may be conservative substitutions or non-conservative substitutions, or both, and may be selected based on a number of factors known in the art, including the divergence needed to be achieved, MHC binding, the presence of ortholog amino acids at the site of substitution, surface exposure, and maintaining the 3-D structure of the protein for optimal processing and presentation.

B-2. Immunogenic PSA Polypeptides

In another aspect, the present disclosure provides isolated immunogenic PSA polypeptides. In one embodiment, the isolated immunogenic PSA polypeptide is a polypeptide consisting of the amino acid sequence of SEQ ID NO:15 or amino acids 4-263 of SEQ ID NO: 15, or a variant thereof. In another embodiment, the isolated immunogenic PSA polypeptide is a polypeptide consisting of the amino acid sequence of SEQ ID NO:17 or amino acids 4-240 of SEQ ID NO: 17, or a variant thereof. In a further embodiment, the isolated immunogenic PSA polypeptide is a polypeptide consisting of the amino acid sequence of SEQ ID NO:19 or amino acids 4-281 of SEQ ID NO: 19, or a variant thereof.

C. Nucleic Acid Molecules Encoding Immunogenic PAA Polypeptides

In some aspects, the present disclosure provides nucleic acid molecules that encode immunogenic PAA polypeptides. The nucleic acid molecules can be deoxyribonucleotides (DNA) or ribonucleotides (RNA). Thus, a nucleic acid molecule can comprise a nucleotide sequence disclosed herein wherein thymidine (T) can also be uracil (U), which reflects the differences between the chemical structures of DNA and RNA. The nucleic acid molecules can be modified forms, single or double stranded forms, or linear or circular forms. The nucleic acid molecules can be prepared using methods known in the art light of the present disclosure.

C-1. Nucleic Acid Molecules Encoding Immunogenic PSMA Polypeptides

In one aspect, the present disclosure provides isolated nucleic acid molecules, or degenerate variants thereof, which comprise a nucleotide sequence encoding an immu-

16

nogenic PSMA polypeptide, including the immunogenic PSMA polypeptides provided by the present disclosure or a functional variant thereof.

In some embodiments, the nucleotide sequence encodes a membrane-bound immunogenic PSMA polypeptide. In some particular embodiments, the isolated nucleic acid molecule comprises a nucleotide sequence, or a degenerate variant thereof, selected from the group consisting of:

1) a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:9;

2) a nucleotide sequence encoding amino acids 4-739 of SEQ ID NO:3;

3) a nucleotide sequence encoding amino acids 4-739 of SEQ ID NO:5; and

4) a nucleotide sequence encoding amino acids 4-739 of SEQ ID NO:7.

In some other particular embodiments, the nucleotide sequence encodes a variant of an immunogenic PSMA polypeptide of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, wherein the variant has an amino acid sequence that has (a) 93% to 99% identity with the amino acid sequence of SEQ ID NO:1 and (b) at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity with the amino acid sequence of SEQ ID NO: 3, 5, or 7.

In still some other particular embodiments, the isolated nucleic acid molecule comprises a nucleotide sequence, or a degenerate variant thereof, selected from the group consisting of:

1) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:4;

2) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:6;

3) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:8; and

4) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:10.

C-2. Nucleic Acid Molecules Encoding Immunogenic PSA Polypeptides

In another aspect, the present disclosure provides isolated nucleic acid molecules, or degenerate variants thereof, which encode an immunogenic PSA polypeptide, including the immunogenic PSA polypeptides provided by the present disclosure.

In some embodiments, the isolated nucleic acid molecule comprises or consists of a nucleotide sequence encoding a cytosolic immunogenic PSA polypeptide. In one embodiment, the nucleotide sequence encodes a cytosolic immunogenic PSA polypeptide consisting of consecutive amino acid residues 4-240 of SEQ ID NO:17. In another embodiment, the nucleotide sequence encodes a cytosolic immunogenic PSA polypeptide comprising the amino acid sequence of SEQ ID NO:17. In still another embodiment, the nucleotide sequence encodes a cytosolic immunogenic PSA polypeptide consisting of the amino acid sequence of SEQ ID NO:17. In yet another embodiment, the nucleotide sequence encodes a functional variant of any of said cytosolic immunogenic polypeptides provided herein above.

In some other embodiments, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a membrane-bound immunogenic PSA polypeptide. In one embodiment, the nucleotide sequence encodes a membrane-bound immunogenic PSA polypeptide comprising consecutive amino acid residues 4-281 of SEQ ID NO:19. In another embodiment, the nucleotide sequence encodes a membrane-bound immunogenic PSA polypeptide comprising the amino acid sequence of SEQ ID NO:19. In still another embodiment, the nucleotide sequence encodes a membrane-bound

60 membrane-bound immunogenic PSA polypeptide comprising the amino acid sequence of SEQ ID NO:19. In still another embodiment, the nucleotide sequence encodes a membrane-bound

17

immunogenic PSA polypeptide consisting of the amino acid sequence of SEQ ID NO:19. In yet other embodiments, the nucleotide sequence encodes a functional variant of any of said membrane-bound immunogenic PSA polypeptides provided herein above.

Examples of particular nucleic acid molecules provided by the present disclosure include:

- 1) a nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO: 18;
- 2) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO; 18;
- 3) a nucleic acid molecule consisting of the nucleotide sequence of SEQ ID NO; 20;
- 4) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 20; and
- 5) a degenerate variant of any of the nucleic acid molecules 1)-4) above.

C-3. Nucleic Acid Molecules Encoding Two or More Immunogenic PAA Polypeptides

In another aspect, the present disclosure provides a nucleic acid molecule that encodes more than one immunogenic PAA polypeptide, for example at least two, at least three, or at least four immunogenic PAA polypeptides. Such nucleic acid molecules are also be referred to as “multi-antigen constructs,” “multi-antigen vaccine,” “multi-antigen plasmid,” and the like, in the present disclosure. Thus, in some aspects, one nucleic acid molecule carries two coding nucleotide sequences wherein each of the coding nucleotide sequences expresses an individual immunogenic PAA polypeptide. Such a nucleic acid molecule is also referred to as “dual antigen construct,” “dual antigen vaccine,” or “dual antigen plasmid,” etc., in this disclosure. In some other aspects, one nucleic acid molecule carries three coding nucleotide sequences wherein each of the coding nucleotide sequences expresses an individual immunogenic PAA polypeptide. Such a nucleic acid molecule is also referred to as “triple antigen construct,” “triple antigen vaccine,” or “triple antigen plasmid” in this disclosure. The individual PAA polypeptides encoded by a multi-antigen construct may be immunogenic against the same antigen, such as PSMA, PSA, or PSCA. The individual PAA polypeptides encoded by a multi-antigen construct may be immunogenic against different antigens, for example, one PAA polypeptide being a PSMA polypeptide and another one a PSA polypeptide. Specifically, one multi-antigen construct may encode two or more immunogenic PAA polypeptides in any one of the following combinations:

- 1) at least one immunogenic PSMA polypeptide and at least one immunogenic PSA polypeptide;
- 2) at least one immunogenic PSMA polypeptide and at least one immunogenic PSCA polypeptide;
- 3) at least one immunogenic PSA polypeptide and at least one immunogenic PSCA polypeptide; and
- 4) at least one immunogenic PSMA polypeptide, at least one immunogenic PSA polypeptide, and at least one immunogenic PSCA polypeptide.

The immunogenic PSMA polypeptides encoded by a multi-antigen construct may be either cytosolic, secreted, or membrane-bound, but preferably membrane-bound. Similarly, the immunogenic PSA polypeptide encoded by a multi-antigen construct may be either cytosolic, secreted, or membrane-bound, but preferably cytosolic. The immunogenic PSCA polypeptide encoded by a multi-antigen construct is preferably the full length human PSCA protein, the amino acid sequence of which is set forth in SEQ ID No:21.

In some embodiments, the present disclosure provides a multi-antigen construct that encodes at least one membrane-

18

bound immunogenic PSMA polypeptide and at least one membrane-bound immunogenic PSA polypeptide.

In some other embodiments, the present disclosure provides a multi-antigen construct that encodes at least one membrane-bound immunogenic PSMA polypeptide, at least one cytosolic immunogenic PSA polypeptide, and at least one immunogenic PSCA polypeptide, wherein the at least one cytosolic immunogenic PSA polypeptide comprises amino acids 4-240 of SEQ ID NO:17, wherein the at least one immunogenic PSCA polypeptide is the full length human PSCA protein of SEQ ID NO:21, and wherein the at least one immunogenic PSMA polypeptide is selected from the group consisting of:

- 1) a polypeptide comprising amino acids 15-750 of SEQ ID NO: 1;
- 2) a polypeptide comprising the amino acid sequence of SEQ ID NO:3;
- 3) a polypeptide comprising the amino acid sequence of SEQ ID NO:5;
- 4) a polypeptide comprising the amino acid sequence of SEQ ID NO:7;
- 5) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:9;
- 6) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:3;
- 7) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:5;
- 8) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:7; and
- 9) polypeptide comprising the amino acid sequence of SEQ ID NO: 9.

In some particular embodiments, the present disclosure provides a multi-antigen construct comprising at least one nucleotide sequence encoding an immunogenic PSMA polypeptide, at least one nucleotide sequence encoding an immunogenic PSA polypeptide, and at least one nucleotide sequence encoding an immunogenic PSCA polypeptide, wherein the nucleotide sequence encoding the immunogenic PSA polypeptide is selected from the nucleotide sequence of SEQ ID NO: 18 or SEQ ID NO: 20, wherein the nucleotide sequence encoding the immunogenic PSCA polypeptide is set forth in SEQ ID NO:22, and wherein the nucleotide sequence encoding the immunogenic PSMA polypeptide is selected from the group consisting of:

- 1) the nucleotide sequence of SEQ ID NO:2;
- 2) the nucleotide sequence of SEQ ID NO:4;
- 3) the nucleotide sequence of SEQ ID NO:6;
- 4) the nucleotide sequence of SEQ ID NO:8;
- 5) the nucleotide sequence of SEQ ID NO:10;
- 6) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:4;
- 7) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:6;
- 8) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:8; and
- 9) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:10.

Examples of specific multi-antigen constructs provided by the present disclosure include the nucleic acid molecules that comprise a nucleotide sequence set forth in SEQ ID NOS:23-36.

Multi-antigen constructs provided by the present disclosure can be prepared using various techniques known in the art in light of the disclosure. For example, a multi-antigen construct can be constructed by incorporating multiple independent promoters into a single plasmid (Huang, Y., Z. Chen, et al. (2008). “Design, construction, and character-

ization of a dual-promoter multigenic DNA vaccine directed against an HIV-1 subtype C/B' recombinant." *J Acquir Immune Defic Syndr* 47(4): 403-411; Xu, K., Z. Y. Ling, et al. (2011). "Broad humoral and cellular immunity elicited from a bivalent DNA vaccine encoding HA and NP genes from an H5N1 virus." *Viral Immunol* 24(1): 45-56). The plasmid can be engineered to carry multiple expression cassettes, each consisting of a) a eukaryotic promoter for initiating RNA polymerase dependent transcription, with or without an enhancer element, b) a gene encoding a target antigen, and c) a transcription terminator sequence. Upon delivery of the plasmid to the transfected cell nucleus, transcription will be initiated from each promoter, resulting in the production of separate mRNAs, each encoding one of the target antigens. The mRNAs will be independently translated, thereby producing the desired antigens.

Multi-antigen constructs provided by the present disclosure can also be constructed using a single vector through the use of viral 2A-like polypeptides (Szymczak, A. L. and D. A. Vignali (2005). "Development of 2A peptide-based strategies in the design of multicistronic vectors." *Expert Opin Biol Ther* 5(5): 627-638; de Felipe, P., G. A. Luke, et al. (2006). "E unum pluribus: multiple proteins from a self-processing polyprotein." *Trends Biotechnol* 24(2): 68-75; Luke, G. A., P. de Felipe, et al. (2008). "Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes." *J Gen Virol* 89 (Pt 4): 1036-1042; Ibrahimi, A., G. Vande Velde, et al. (2009). "Highly efficient multicistronic lentiviral vectors with peptide 2A sequences." *Hum Gene Ther* 20(8): 845-860; Kim, J. H., S. R. Lee, et al. (2011). "High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice." *PLoS One* 6(4): e18556). These polypeptides, also called cleavage cassettes or CHYSELs (*cis*-acting hydrolase elements), are approximately 20 amino acids long with a highly conserved carboxy terminal D-V/I-EXNPGP motif (FIG. 2). The cassettes are rare in nature, most commonly found in viruses such as Foot-and-mouth disease virus (FMDV), Equine rhinitis A virus (ERAV), Encephalomyocarditis virus (EMCV), Porcine teschovirus (PTV), and Thosea asigna virus (TAV) (Luke, G. A., P. de Felipe, et al. (2008). "Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes." *J Gen Virol* 89 (Pt 4): 1036-1042). With a 2A-based multi-antigen expression strategy, genes encoding multiple target antigens can be linked together in a single open reading frame, separated by 2A cassettes. The entire open reading frame can be cloned into a vector with a single promoter and terminator. Upon delivery of the constructs to a host cell, mRNA encoding the multiple antigens will be transcribed and translated as a single polyprotein. During translation of the 2A cassettes, ribosomes skip the bond between the C-terminal glycine and proline. The ribosomal skipping acts like a cotranslational autocatalytic "cleavage" that releases upstream from downstream proteins. The incorporation of a 2A cassette between two protein antigens results in the addition of ~20 amino acids onto the C-terminus of the upstream polypeptide and 1 amino acid (proline) to the N-terminus of downstream protein. In an adaptation of this methodology, protease cleavage sites can be incorporated at the N terminus of the 2A cassette such that ubiquitous proteases will cleave the cassette from the upstream protein (Fang, J., S. Yi, et al. (2007). "An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies in vivo." *Mol Ther* 15(6): 1153-1159).

Another strategy for constructing the multi-antigen constructs provided by the present disclosure involves the use of

an internal ribosomal entry site, or IRES. Internal ribosomal entry sites are RNA elements (FIG. 3) found in the 5' untranslated regions of certain RNA molecules (Bonnal, S., C. Boutonnet, et al. (2003). "IRESdb: the Internal Ribosome Entry Site database." *Nucleic Acids Res* 31(1): 427-428). They attract eukaryotic ribosomes to the RNA to facilitate translation of downstream open reading frames. Unlike normal cellular 7-methylguanosine cap-dependent translation, IRES-mediated translation can initiate at AUG codons far within an RNA molecule. The highly efficient process can be exploited for use in multi-cistronic expression vectors (Bochkov, Y. A. and A. C. Palmenberg (2006). "Translational efficiency of EMCV IRES in bicistronic vectors is dependent upon IRES sequence and gene location." *Bio-techniques* 41(3): 283-284, 286, 288). Typically, two transgenes are inserted into a vector between a promoter and transcription terminator as two separate open reading frames separated by an IRES. Upon delivery of the constructs to a host cell, a single long transcript encoding both transgenes will be transcribed. The first ORF will be translated in the traditional cap-dependent manner, terminating at a stop codon upstream of the IRES. The second ORF will be translated in a cap-independent manner using the IRES. In this way, two independent proteins can be produced from a single mRNA transcribed from a vector with a single expression cassette.

Although the multi-antigen expression strategies are described here in the context of a DNA vaccine construct, the principles apply similarly in the context of viral vector genetic vaccines.

D. Vectors Containing a Nucleic Acid Molecule Encoding an Immunogenic PAA Polypeptide

Another aspect of the invention relates to vectors containing one or more nucleic acid molecules of the invention. The vectors are useful for cloning or expressing the immunogenic PAA polypeptides encoded by the nucleic acid molecules, or for delivering the nucleic acid molecule in a composition, such as a vaccine, to a host cell or to a host animal, such as a human. A wide variety of vectors may be prepared to contain and express a nucleic acid molecule of the invention, such as plasmid vectors, cosmid vectors, phage vectors, and viral vectors.

In some embodiments, the disclosure provides a plasmid-based vector containing a nucleic acid molecule of the invention. Representative examples of suitable plasmid vectors include pBR325, pUC18, pSKF, pET23D, and pGB-2. Other representative examples of plasmid vectors, as well as method of constructing such vectors, are described in U.S. Pat. Nos. 5,580,859, 5,589,466, 5,688,688, 5,814,482 and 5,580,859.

In other embodiments, the present invention provides vectors that are constructed from viruses, such as retroviruses, alphaviruses, adenoviruses. Representative examples of retroviral vectors are described in more detail in EP 0,415,731; PCT Publication Nos. WO 90/07936; WO 91/0285, WO 9311230; WO 9310218, WO 9403622; WO 9325698; WO 9325234; and U.S. Pat. Nos. 5,219,740, 5,716,613, 5,851,529, 5,591,624, 5,716,826, 5,716,832, and 5,817,491. Representative examples of vectors that can be generated from alphaviruses are described in U.S. Pat. Nos. 5,091,309 and 5,217,879, 5,843,723, and 5,789,245. In some particular embodiments, the present disclosure provides adenoviral vectors derived from non-human primate adenoviruses, such as simian adenoviruses. Examples of such adenoviral vectors, as well as their preparation, are

described in PCT application publication WO2005/071093 and WO 2010/086189, and include non-replicating vectors such as ChAd3, ChAd4, ChAd5, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63, ChAd68, ChAd82, ChAd55, ChAd73, ChAd83, ChAd146, ChAd147, PanAd1, Pan Ad2, and Pan Ad3, and replication-competent vectors such as Ad4 and Ad7 vectors. It is preferred that in constructing the adenoviral vectors from the simian adenoviruses one or more of the early genes from the genomic region of the virus selected from E1A, E1B, E2A, E2B, E3, and E4 are either deleted or rendered non-functional by deletion or mutation. In a particular embodiment, the vector is constructed from ChAd3 or ChAd68. Suitable vectors can also be generated from other viruses such as: pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch et al., PNAS 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Pat. Nos. 4,603,112, 4,769,330 and 5,017,487; WO 89/01973); adeno-associated vectors (see, e.g., U.S. Pat. No. 5,872,005); SV40 (Mulligan et al., Nature 277:108-114, 1979); herpes (Kit, Adv. Exp. Med. Biol. 215:219-236, 1989; U.S. Pat. No. 5,288,641); and lentivirus such as HIV (Poznansky, J. Virol. 65:532-536, 1991).

Methods of constructing vectors are well known in the art. Expression vectors typically include one or more control elements that are operatively linked to the nucleic acid sequence to be expressed. The term "control elements" refers collectively to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription, and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present so long as the selected coding sequence is capable of being replicated, transcribed, and translated in an appropriate host cell. The control elements are selected based on a number of factors known to those skilled in that art, such as the specific host cells and source or structures of other vector components. For enhancing the expression of an immunogenic PAA polypeptide, a Kozak sequence can be provided upstream of the sequence encoding the immunogenic PAA polypeptide. For vertebrates, a known Kozak sequence is (GCC)NC-CATGG, wherein N is A or G and GCC is less conserved. Exemplary Kozak sequences that can be used include ACCAUGG and ACCATGG.

E. Compositions Comprising an Immunogenic PAA Polypeptide (Polypeptide Compositions)

In another aspect, the present disclosure provides compositions comprising one or more isolated immunogenic PAA polypeptides provided by the present disclosure ("polypeptide composition"). In some embodiments, the polypeptide composition is an immunogenic composition useful for eliciting an immune response against a PAA protein in a mammal, such as a mouse, dog, nonhuman primates or human. In some other embodiments, the polypeptide composition is a vaccine composition useful for immunization of a mammal, such as a human, for inhibiting abnormal cell proliferation, for providing protection against the development of cancer (used as a prophylactic), or for treatment of disorders (used as a therapeutic) associated with PAA over expression, such as cancers, particularly prostate cancer.

A polypeptide composition provided by the present disclosure may contain a single type of immunogenic PAA polypeptide, such as an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, or an immunogenic PSCA polypeptide. A composition may also contain a combination of two or more different types of immunogenic PAA polypeptides. For example, a polypeptide composition may contain immunogenic PAA polypeptides in any of the following combinations:

- 10 1) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide;
- 2) an immunogenic PSMA polypeptide and a PSCA polypeptide; or
- 3) an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and a PSCA polypeptide.

An immunogenic composition or vaccine composition provided by the present disclosure may further comprise a pharmaceutically acceptable excipient. Pharmaceutically acceptable excipients for immunogenic or vaccine compositions are known in the art. Examples of suitable excipients include biocompatible oils, such as rape seed oil, sunflower oil, peanut oil, cotton seed oil, jojoba oil, squalan, squalene, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, pH modifiers, and anti-oxidative agents.

The immunogenic PAA polypeptide in a composition, particularly an immunogenic composition or a vaccine composition, may be linked to, conjugated to, or otherwise incorporated into a carrier for administration to a recipient. The term "carrier" refers to a substance or structure that an immunogenic polypeptide can be attached to or otherwise associated with for delivery of the immunogenic polypeptide to the recipient (e.g., patient). The carrier itself may be immunogenic. Examples of carriers include immunogenic polypeptides, immune CpG islands, limpet hemocyanin (KLH), tetanus toxoid (TT), cholera toxin subunit B (CTB), bacteria or bacterial ghosts, liposome, chitosome, virosomes, microspheres, dendritic cells, or their like. One or more immunogenic PAA polypeptide molecules may be linked to a single carrier molecule. Methods for linking an immunogenic polypeptide to a carrier are known in the art,

A vaccine composition or immunogenic composition provided by the present disclosure may be used in conjunction with one or more immune modulators or adjuvants. The immune modulators or adjuvants may be formulated separately from the vaccine composition, or they may be part of the same vaccine composition formulation. Thus, in one embodiment, the vaccine composition further comprises one or more immune modulators or adjuvants. Examples of immune modulators and adjuvants are provided herein below.

The polypeptide compositions, including the immunogenic and vaccine compositions, can be prepared in any suitable dosage forms, such as liquid forms (e.g., solutions, suspensions, or emulsions) and solid forms (e.g., capsules, tablets, or powder), and by methods known to one skilled in the art.

F. Compositions Comprising an Immunogenic PAA Nucleic Acid Molecule (Nucleic Acid Compositions)

The present disclosure also provides a composition comprising an isolated nucleic acid molecule or vector provided by the present disclosure (herein "nucleic acid composi-

23

tion'). The nucleic acid compositions are useful for eliciting an immune response against a PAA protein in vitro or in vivo in a mammal, including a human.

In some particular embodiments, the nucleic acid composition is a DNA vaccine composition for administration to humans for inhibiting abnormal cell proliferation, providing protection against the development of cancer (used as a prophylactic), or for treatment of cancer (used as a therapeutic) associated with PAA over-expression, or for eliciting an immune response to a particular human PAA, such as PSMA, PSA, and PSCA. The nucleic acid molecule in the composition may be a "naked" nucleic acid molecule, i.e. simply in the form of an isolated DNA free from elements that promote transfection or expression. Alternatively, the nucleic acid molecule in the composition can be incorporated into a vector.

A nucleic acid composition provided by the present disclosure may comprise individual isolated nucleic acid molecules that each encode only one type of immunogenic PAA polypeptide, such as an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, or an immunogenic PSCA polypeptide.

A nucleic acid composition may comprise a multi-antigen construct provided by the present disclosure that encodes two or more types of immunogenic PAA polypeptides. A multi-antigen construct may encode two or more immunogenic PAA polypeptides in any of the following combinations:

- 1) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide;
- 2) an immunogenic PSMA polypeptide and an immunogenic PSCA polypeptide;
- 3) an immunogenic PSA polypeptide and an immunogenic PSCA polypeptide; and
- 4) an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide.

The nucleic acid compositions, including the DNA vaccine compositions, may further comprise a pharmaceutically acceptable excipient. Examples of suitable pharmaceutically acceptable excipients for nucleic acid compositions, including DNA vaccine compositions, are well known to those skilled in the art and include sugars, etc. Such excipients may be aqueous or non aqueous solutions, suspensions, and emulsions. Examples of non-aqueous excipients include propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Examples of aqueous excipient include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Suitable excipients also include agents that assist in cellular uptake of the polynucleotide molecule. Examples of such agents are (i) chemicals that modify cellular permeability, such as bupivacaine, (ii) liposomes or viral particles for encapsulation of the polynucleotide, or (iii) cationic lipids or silica, gold, or tungsten microparticles which associate themselves with the polynucleotides. Anionic and neutral liposomes are well-known in the art (see, e.g., *Liposomes: A Practical Approach*, RPC New Ed, IRL press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides. Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include Lipofectin™ also known as DOTMA (N-[I-(2,3-dioleyloxy) propyl N,N, N-trimethylammonium chloride), DOTAP (1,2-bis (oleyl-oxy)-3 (trimethylammonio) propane), DDAB (dimethyl-di-octadecyl-ammonium bromide), DOGS (di-octadecylamido-glycyl spermine) and cholesterol derivatives such as

24

DCChol (3 beta-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Pat. No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Pat. No. 5,527,928. A particular useful cationic lipid formulation that may be used with the nucleic vaccine provided by the disclosure is VAXFFECTIN, which is a commixture of a cationic lipid (GAP-DMORIE) and a neutral phospholipid (DPyPE) which, when combined in an aqueous vehicle, self-assemble to form liposomes. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPPE (dioleyl phosphatidylethanolamine), as described in WO 90/11092 as an example. In addition, a DNA vaccine can also be formulated with a nonionic block copolymer such as CRL1005.

G. Uses of the Immunogenic PAA Polypeptides, Nucleic Acid Molecules, and Compositions

In other aspects, the present disclosure provides methods of using the immunogenic PAA polypeptides, isolated nucleic acid molecules, and compositions comprising an immunogenic PAA polypeptide or isolated nucleic acid molecule described herein above.

In one aspect, the present disclosure provides a method of eliciting an immune response against a PAA in a mammal, particularly a human, comprising administering to the mammal an effective amount of (1) an immunogenic PAA polypeptide provided by the disclosure that is immunogenic against the target PAA, (2) an isolated nucleic acid molecule encoding such an immunogenic PAA polypeptide, (3) a composition comprising such an immunogenic PAA polypeptide, or (4) a composition comprising an isolated nucleic acid molecule encoding such an immunogenic PAA polypeptide.

In some embodiments, the disclosure provides a method of eliciting an immune response against PSMA in a human, comprising administering to the human an effective amount of an immunogenic PSMA composition provided by the present disclosure, wherein the immunogenic PSMA composition is selected from: (1) an immunogenic PSMA polypeptide, (2) an isolated nucleic acid molecule encoding an immunogenic PSMA polypeptide, (3) a composition comprising an immunogenic PSMA polypeptide, or (4) a composition comprising an isolated nucleic acid molecule encoding an immunogenic PSMA polypeptide.

In some other embodiments, the disclosure provides a method of eliciting an immune response against PSA in a human, comprising administering to the human an effective amount of an immunogenic PSA composition provided by the present disclosure, wherein the immunogenic PSA composition is selected from: (1) an immunogenic PSA polypeptide, (2) an isolated nucleic acid molecule encoding an immunogenic PSA polypeptide, (3) a composition comprising an immunogenic PSA polypeptide, or (4) a composition comprising an isolated nucleic acid molecule encoding an immunogenic PSA polypeptide.

In another aspect, the present disclosure provides a method of inhibiting abnormal cell proliferation in a human, wherein the abnormal cell proliferation is associated with over-expression of a PAA. The method comprises administering to the human an effective amount of immunogenic PAA composition provided by the present disclosure that is immunogenic against the over-expressed PAA. The immunogenic PAA composition may be (1) an immunogenic PAA polypeptide, (2) an isolated nucleic acid molecule encoding one or more immunogenic PAA polypeptides, (3) a com-

25

sition comprising an immunogenic PAA polypeptide, or (4) a composition comprising an isolated nucleic acid molecule encoding one or more immunogenic PAA polypeptides. In some embodiments, the method is for inhibiting abnormal cell proliferation in prostate in a human. In a particular embodiment, the present disclosure provide a method of inhibiting abnormal cell proliferation in prostate over-expressing PSMA, comprising administering to the human effective amount of (1) an immunogenic PSMA polypeptide, (2) an isolated nucleic acid molecule encoding one or more immunogenic PSMA polypeptides, (3) a composition comprising an immunogenic PSMA polypeptide, or (4) a composition comprising an isolated nucleic acid molecule encoding one or more immunogenic PSMA polypeptide.

In another aspect, the present disclosure provides a method of treating cancer in a human wherein cancer is associated with over-expression of a PAA. The method comprises administering to the human an effective amount of immunogenic PAA composition capable of eliciting an immune response against the over-expressed PAA. The immunogenic PAA composition may be (1) an immunogenic PAA polypeptide, (2) an isolated nucleic acid molecule encoding one or more immunogenic PAA polypeptides, (3) a composition comprising an immunogenic PAA polypeptide, or (4) a composition comprising an isolated nucleic acid molecule encoding one or more immunogenic PAA polypeptides. Examples of cancers that may be treated with the method include breast cancer, stomach cancer, ovarian cancer, lung cancer, bladder cancer, colorectal cancer, renal cancer, pancreatic cancer and prostate cancer.

In some embodiments, the disclosure provides a method of treating prostate cancer in a human, comprising administering to the human an effective amount of a nucleic acid composition provided herein above. The nucleic acids in the composition may encode only one particular immunogenic PAA polypeptide, such as an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, or an immunogenic PSCA polypeptide. The nucleic acids in the composition may also encode two or more different immunogenic PAA polypeptides, such as: (1) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide; (2) an immunogenic PSMA polypeptide and an immunogenic PSCA polypeptide; (3) an immunogenic PSA polypeptide and an immunogenic PSCA polypeptide; (4) an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide. Each individual nucleic acid molecule in the composition may encode only one particular immunogenic PAA polypeptide, such as a PSMA polypeptide, a PSA polypeptide, or a PSCA polypeptide. Alternatively, an individual nucleic acid molecule in the composition may be a multi-antigen constructs encoding two different types of immunogenic PAA polypeptides, such as: (1) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide; (2) an immunogenic PSMA polypeptide and an immunogenic PSCA polypeptide; (3) an immunogenic PSCA polypeptide and an immunogenic PSA polypeptide; or (4) an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide. In some particular embodiments, the nucleic acid composition comprises a multi-antigen construct that encode at least (4) an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide. The immunogenic PSCA polypeptide contained in vaccine compositions or expressed by a nucleic acid in vaccine compositions for the treatment of prostate cancer in human is preferably the human full length PSCA protein.

26

The polypeptide and nucleic acid compositions can be administered to an animal, including human, by a number of methods known in the art. Examples of suitable methods include: (1) intramuscular, intradermal, intraepidermal, intravenous, intraarterial, subcutaneous, or intraperitoneal administration, (2) oral administration, and (3) topical application (such as ocular, intranasal, and intravaginal application). One particular method of intradermal or intraepidermal administration of a nucleic acid vaccine composition that may be used is gene gun delivery using the Particle Mediated Epidermal Delivery (PMED™) vaccine delivery device marketed by PowderMed. PMED is a needle-free method of administering vaccines to animals or humans. The PMED system involves the precipitation of DNA onto microscopic gold particles that are then propelled by helium gas into the epidermis. The DNA-coated gold particles are delivered to the APCs and keratinocytes of the epidermis, and once inside the nuclei of these cells, the DNA elutes off the gold and becomes transcriptionally active, producing encoded protein. This protein is then presented by the APCs to the lymphocytes to induce a T-cell-mediated immune response. Another particular method for intramuscular administration of a nucleic acid vaccine provided by the present disclosure is electroporation. Electroporation uses controlled electrical pulses to create temporary pores in the cell membrane, which facilitates cellular uptake of the nucleic acid vaccine injected into the muscle. Where a CpG is used in combination with a nucleic acid vaccine, it is preferred that the CpG and nucleic acid vaccine are co-formulated in one formulation and the formulation is administered intramuscularly by electroporation.

The effective amount of the immunogenic PAA polypeptide or nucleic acid encoding an immunogenic PAA polypeptide in the composition to be administered in a given method provided by the present disclosure can be readily determined by a person skilled in the art and will depend on a number of factors. In a method of treating cancer, such as prostate cancer, factors that may be considered in determining the effective amount of the immunogenic PAA polypeptide or nucleic acid include, but not limited: (1) the subject to be treated, including the subject's immune status and health, (2) the severity or stage of the cancer to be treated, (3) the specific immunogenic PAA polypeptides used or expressed, (4) the degree of protection or treatment desired, (5) the administration method and schedule, and (6) other therapeutic agents (such as adjuvants or immune modulators) used. In the case of nucleic acid vaccine compositions, including the multi-antigen vaccine compositions, the method of formulation and delivery are among the key factors for determining the dose of the nucleic acid required to elicit an effective immune response. For example, the effective amounts of the nucleic acid may be in the range of 2 µg/dose-10 mg/dose when the nucleic acid vaccine composition is formulated as an aqueous solution and administered by hypodermic needle injection or pneumatic injection, whereas only 16 ng/dose-16 µg/dose may be required when the nucleic acid is prepared as coated gold beads and delivered using a gene gun technology. The dose range for a nucleic acid vaccine by electroporation is generally in the range of 0.5-10 mg/dose. In the case where the nucleic acid vaccine is administered together with a CpG by electroporation in a co-formulation, the dose of the nucleic acid vaccine may be in the range of 0.5-5 mg/dose and the dose of CpG is typically in the range of 0.05 mg-5 mg/dose, such as 0.05, 0.2, 0.6, or 1.2 mg/dose per person.

The nucleic acid or polypeptide vaccine composition of the present invention can be used in a prime-boost strategy

to induce robust and long-lasting immune response. Priming and boosting vaccination protocols based on repeated injections of the same immunogenic construct are well known. In general, the first dose may not produce protective immunity, but only "primes" the immune system. A protective immune response develops after the second or third dose (the "boosts"). The boosts are performed according to conventional techniques, and can be further optimized empirically in terms of schedule of administration, route of administration, choice of adjuvant, dose, and potential sequence when administered with another vaccine. In one embodiment, the nucleic acid or polypeptide vaccines of the present invention are used in a conventional homologous prime-boost strategy, in which the same vaccine is administered to the animal in multiple doses. In another embodiment, the nucleic acid or polypeptide vaccine compositions are used in a heterologous prime-boost vaccination, in which different types of vaccines containing the same antigens are administered at predetermined time intervals. For example, a nucleic acid construct may be administered in the form of a plasmid in the initial dose ("prime") and as part of a vector in the subsequent doses ("boosts"), or vice versa.

For the treatment of prostate cancer, the polypeptide or nucleic acid vaccines of the present invention may be used together with prostate cancer vaccines based on other antigens, such as prostatic acid phosphatase-based antigens and androgen receptor.

The polypeptide or nucleic acid vaccine composition of the present invention may be used together with one or more adjuvants. Examples of suitable adjuvants include: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl polypeptides or bacterial cell wall components), such as for example (a) MF59TM (PCT Publication No. WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80 (polyoxyethylene sorbitan monoleate), and 0.5% Span 85 (sorbitan trioleate) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBITTM adjuvant system (RAS) (Ribi Immunochem, Hamilton, Mont.) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS); (2) saponin adjuvants, such as QS21, STIMULONTM (Cambridge Bioscience, Worcester, Mass.), Abisco[®] (Isconova, Sweden), or Iscomatrix[®] (Commonwealth Serum Laboratories, Australia); (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (PCT Publication No. WO 99/44636), etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL), optionally in the substantial absence of alum when used with pneumococcal saccharides (e.g. GB-2220221, EP-A-0689454, WO 00/56358); (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (e.g. EP-A-0835318, EP-A-0735898, EP-A-0761231); (7) oligonucleotides comprising CpG motifs, i.e. containing at least one CG dinucleotide, where the cytosine is unmethylated (e.g., Krieg, *Vaccine* (2000) 19:618-622; Krieg, *Curr Opin Mol Ther* (2001) 3:15-24; WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581); (8) a

polyoxyethylene ether or a polyoxyethylene ester (e.g. WO 99/52549); (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (e.g., WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (e.g., WO 01/21152); (10) a saponin and an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) (e.g., WO 00/62800); (11) metal salt including aluminum salts (such as alum, aluminum phosphate, aluminum hydroxide); (12) a saponin and an oil-in-water emulsion (e.g. WO 99/11241); (13) a saponin (e.g. QS21)+3dMPL+ IM2 (optionally+a sterol)(e.g. WO 98/57659); (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition, such as Muramyl polypeptides including N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), (15) ligands for toll-like receptors (TLR), natural or synthesized (e.g. Kanzler et al., *Nature Med.* 13:1552-1559 (2007)), including TLR3 ligands such as polyI:C and similar compounds such as Hiltonol and Ampligen.

The polypeptide or nucleic acid vaccine compositions of the present invention may be used together with one or more immune modulators. Examples of suitable immune modulators include protein tyrosine kinase inhibitors (such as afatinib, axitinib, cediranib, erlotinib, gefitinib, grandinin, lapatinib, lestaurtinib, neratinib, pazopanib, quizartinib, regorafenib, semaxanib, sorafenib, sunitinib, tivozanib, toceranib, bosutinib and vandetanib), CD40 agonists (such as CD40 agonist antibody), OX40 agonists (such as OX40 agonist antibody), CTLA-4 inhibitors (such as anti-CTLA-4 antibody Ipilimumab and Tremelimumab), TLR agonists, 4-1BB agonists, Tim-1 antagonists, LAGE-3 antagonists and PD-L1 & PD-1 antagonists.

H. Vaccine-Based Immunotherapy Regimens (VBIR)

In a further aspect, the present disclosure provides a method of enhancing the immunogenicity or therapeutic effect of a vaccine for the treatment of a neoplastic disorder in a mammal, particularly a human. The method comprises administering to the mammal receiving the vaccine for the treatment of a neoplastic disorder (1) an effective amount of at least one immune-suppressive-cell inhibitor (ISC inhibitor) and (2) an effective amount of at least one immune-effector-cell enhancer (IEC enhancer). The method may be used in combination with a vaccine in any form or formulation, for example, a subunit vaccine, a protein-based vaccine, a peptide-based vaccine, or a nucleic acid-based vaccines such as a DNA-based vaccine, a RNA-based vaccine, a plasmid-based vaccine, or a vector-based vaccine. In addition, the method is not limited to any particular types of vaccines or any particular types of cancer. Rather, the method may be used in combination with any vaccine intended for the treatment of neoplastic disorder, including benign, pre-malignant, and malignant neoplastic disorders. For example, the method may be used in combination a vaccine that is intended for the treatment of any of the following neoplastic disorders: carcinoma including that of the bladder (including accelerated and metastatic bladder cancer), breast, colon (including colorectal cancer), kidney, liver, lung (including small and non-small cell lung cancer and lung adenocarcinoma), ovary, prostate, testes, genitourinary tract, lymphatic system, rectum, larynx, pancreas

(including exocrine pancreatic carcinoma), esophagus, stomach, gall bladder, cervix, thyroid, and skin (including squamous cell carcinoma); hematopoietic tumors of lymphoid lineage including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, histiocytic lymphoma, and Burkitts lymphoma; hematopoietic tumors of myeloid lineage including acute and chronic myelogenous leukemias, myelodysplastic syndrome, myeloid leukemia, and promyelocytic leukemia; tumors of the central and peripheral nervous system including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; other tumors including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, and teratocarcinoma; melanoma, unresectable stage III or IV malignant melanoma, squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal cancer, renal cancer, ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer, gastric cancer, germ cell tumor, bone cancer, bone tumors, adult malignant fibrous histiocytoma of bone; childhood malignant fibrous histiocytoma of bone, sarcoma, pediatric sarcoma, sinonasal natural killer, neoplasms, plasma cell neoplasm; myelodysplastic syndromes; neuroblastoma; testicular germ cell tumor, intraocular melanoma, myelodysplastic syndromes; myelodysplastic/myeloproliferative diseases, synovial sarcoma, chronic myeloid leukemia, acute lymphoblastic leukemia, philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL), multiple myeloma, acute myelogenous leukemia, chronic lymphocytic leukemia, and mastocytosis.

In some embodiments, present disclosure provides a method of enhancing the immunogenicity or therapeutic effect of a vaccine for the treatment of prostate cancer in a human. The vaccine administered may be capable of eliciting an immune response against any human PAA, such as PSMA, PSA, or PSCA. In some particular embodiments, the vaccine administered comprises a nucleic acid molecule encoding an antigen capable of eliciting immunogenicity against a human PAA, such as PSMA, PSA, or PSCA. Examples of specific nucleic acid molecules that may be contained in the vaccine include the following provided by the present disclosure:

1) a nucleic acid molecule encoding an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, or an immunogenic PSCA polypeptide;

2) a nucleic acid molecule encoding two immunogenic PAA polypeptides provided by the present disclosure, such as a) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide; b) an immunogenic PSMA polypeptide and an immunogenic PSCA polypeptide; or c) an immunogenic PSA polypeptide and an immunogenic PSCA polypeptide; and

3) a nucleic acid molecule encoding three immunogenic PAA polypeptides, which are an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide.

In another further aspect, the present disclosure provides a method of treating a neoplastic disorder in a mammal, particularly a human. The method comprises administering to the mammal (1) an effective amount of a vaccine capable

of eliciting an immune response against a TAA associated with the neoplastic disorder, (2) an effective amount of at least one immune-suppressive-cell inhibitor (ISC inhibitor), and (3) an effective amount of at least one immune-effector-cell enhancer (IEC enhancer). Any vaccine that is capable of eliciting an immune response against a particular TAA may be used in the method. Many TAAs are known in the art. In addition to the prostate-associated antigens, the following are examples of TAAs that are known in the art: CEA, MUC-1, Ep-CAM, 5T4, hCG-b, K-ras, and TERT for colorectal cancer; CEA, Muc-1, p53, mesothelin, Survivin, and NY-ESO-1 for ovarian cancer; Muc-1, 5T4, WT-1, TERT, CEA, EGF-R and MAGE-A3 for non-small cell lung cancer; 5T4 for renal cell carcinoma; and Muc-1, mesothelin, K-Ras, Annexin A2, TERT, and CEA for pancreatic cancer. New TAAs continue to be identified. A vaccine that is capable of eliciting an immune response against any of the known or new TAAs can be used in the method. In addition, the vaccine administered may be in any form or formulation, for example, subunit vaccines, protein-based vaccine, peptide based vaccines, or nucleic acid-based vaccines such DNA-based vaccines, RNA-based vaccines, plasmid-based vaccines, or vector-based vaccines.

In some embodiments, the present disclosure provides a method of treating a prostate cancer in a human, the method comprising administering to the human a vaccine capable of eliciting an immune response against any human PAA, such as PSMA, PSA, or PSCA. In some particular embodiments, the vaccine administered comprises a nucleic acid molecule encoding an antigen capable of eliciting immunogenicity against a human PAA, such as PSMA, PSA, or PSCA. Examples of specific nucleic acid molecules that may be contained in the vaccine include the following provided by the present disclosure:

1) a nucleic acid molecule encoding an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, or an immunogenic PSCA polypeptide;

2) a nucleic acid molecule encoding two immunogenic PAA polypeptides provided by the present disclosure, such as a) an immunogenic PSMA polypeptide and an immunogenic PSA polypeptide; b) an immunogenic PSMA polypeptide and an immunogenic PSCA polypeptide; or c) an immunogenic PSA polypeptide and an immunogenic PSCA polypeptide; and

3) a nucleic acid molecule encoding three immunogenic PAA polypeptides, which are an immunogenic PSMA polypeptide, an immunogenic PSA polypeptide, and an immunogenic PSCA polypeptide.

The method of treating a neoplastic disorder in a mammal and the method of enhancing the immunogenicity or therapeutic effect of a vaccine for the treatment of a neoplastic disorder in a mammal described herein above are alternatively referred to as "vaccine-based immunotherapy regimens" (or "VBIR").

In the vaccine-based immunotherapy regimens, the IEC enhancers and ISC inhibitors may be administered by any suitable methods and routes, including (1) systemic administration such as intravenous, intramuscular, or oral administration, and (2) local administration such intradermal and subcutaneous administration. Where appropriate or suitable, local administration is generally preferred over systemic administration. Local administration of any IEC enhancer and ISC inhibitor can be carried out at any location of the body of the mammal that is suitable for local administration of pharmaceuticals; however, it is more preferable that these immune modulators are administered locally at close proximity to the vaccine draining lymph node.

31

Two or more specific IEC enhancers from a single class of IEC enhancers (for examples, two CTLA-agonists) may be administered in combination with the ISC inhibitors. In addition, two or more specific IEC enhancers from two or more different classes of IEC enhancers (for example, one CTLA-4 antagonist and one TLR agonist) may be administered together. Similarly, two or more specific ISC inhibitors from a single class of ISC inhibitors (for examples, two or more protein kinase inhibitors) may be administered in combination with the IEC enhancers. In addition, two or more specific ISC inhibitors from two or more different classes of ISC inhibitors (for example, one protein kinase inhibitor and one COX-2 inhibitor) may be administered together.

In the vaccine-based immunotherapy regimens the vaccine may be administered simultaneously or sequentially with any or all of the immune modulators (i.e., ISC inhibitors and IEC enhancers) used. Similarly, when two or more immune modulators are used, they may be administered simultaneously or sequentially with respect to each other. In some embodiments, a vaccine is administered simultaneously (e.g., in a mixture) with respect to one immune modulator, but sequentially with respect to one or more additional immune modulators. Co-administration of the vaccine and the immune modulators in the vaccine-based immunotherapy regimen can include cases in which the vaccine and at least one immune modulator are administered so that each is present at the administration site, such as vaccine draining lymph node, at the same time, even though the antigen and the immune modulators are not administered simultaneously. Co-administration of the vaccine and the immune modulators also can include cases in which the vaccine or the immune modulator is cleared from the administration site, but at least one cellular effect of the cleared vaccine or immune modulator persists at the administration site, such as vaccine draining lymph node, at least until one or more additional immune modulators are administered to the administration site. In cases where a nucleic acid vaccine is administered in combination with a CpG, the vaccine and CpG may be contained in a single formulation and administered together by any suitable method. In some embodiments, the nucleic acid vaccine and CpG in a co-formulation (mixture) is administered by intramuscular injection in combination with electroporation.

Any ISC inhibitors may be used in the vaccine-based immunotherapy regimens. Examples of classes of ISC inhibitors include protein kinase inhibitors, cyclooxygenase-2 (COX-2) inhibitors, phosphodiesterase type 5 (PDE5) inhibitors, and DNA crosslinkers. Examples COX-2 inhibitors include celecoxib and rofecoxib. Examples of PDE5 inhibitors include avanafil, lodenafil, mirodenafil, sildenafil, tadalafil, vardenafil, udenafil, and zaprinast. An example of DNA crosslinkers is cyclophosphamide. Examples of specific protein kinase inhibitors are described in details below.

The term “protein kinase inhibitor” refers to any substance that acts as a selective or non-selective inhibitor of a protein kinase. The term “protein kinases” refers to the enzymes that catalyze the transfer of the terminal phosphate of adenosine triphosphate to tyrosine, serine or threonine residues in protein substrates. Protein kinases include receptor tyrosine kinases and non-receptor tyrosine kinases. Examples of receptor tyrosine kinases include EGFR (e.g., EGFR/HER1/ErbB1, HER2/Neu/ErbB2, HER3/ErbB3, HER4/ErbB4), INSR (insulin receptor), IGF-IR, IGF-IIIR, IRR (insulin receptor-related receptor), PDGFR (e.g., PDG-FRA, PDGFRB), c-KIT/SCFR, VEGFR-1/FLT-1, VEGFR-

32

2/FLK-1/KDR, VEGFR-3/FLT-4, FLT-3/FLK-2, CSF-1R, FGFR 1-4, CCK4, TRK A-C, MET, RON, EPHA 1-8, EPHB 1-6, AXL, MER, TYRO3, TIE, TEK, RYK, DDR 1-2, RET, c-ROS, LTK (leukocyte tyrosine kinase), ALK (anaplastic lymphoma kinase), ROR 1-2, MUSK, AATYK 1-3, and RTK 106. Examples of non-receptor tyrosine kinases include BCR-ABL, Src, Frk, Btk, Csk, Ab1, Zap70, Fes/Fps, Fak, Jak, Ack, and LIMK. In the vaccine-based immunotherapy regimen provided by the present disclosure, the 10 protein kinase inhibitors are administered to the mammal at a suboptimal dose. The term “suboptimal dose” refers to the dose amount that is below the minimum effective dose when the tyrosine kinase inhibitor is administered in a monotherapy (i.e., where the protein kinase inhibitor is administered alone without any other therapeutic agents) for the target neoplastic disorder.

Examples of specific protein kinase inhibitors suitable for use in the vaccine-based immunotherapy regimen include Lapatinib, AZD 2171, ET180CH 3, Indirubin-3'-oxime, 20 NSC-154020, PD 169316, Quercetin, Roscovitine, Triciribine, ZD 1839, 5-Iodotubercidin, Adaphostin, Aloisine, Alsterpaullone, Aminogenistein, API-2, Apigenin, Arctigenin, ARRY-334543, Axitinib (AG-013736), AY-22989, AZD 2171, Bisindolylmaleimide IX, CCI-779, Chelerythrine, DMPQ, DRB, Edelfosine, ENMD-981693, Erbstatin analog, Erlotinib, Fasudil, Gefitinib (ZD1839), H-7, H-8, H-89, HA-100, HA-1004, HA-1077, HA-1100, Hydroxyfasudil, Kenpaullone, KN-62, KY12420, LFM-A13, Luteolin, LY294002, LY-294002, Mallotoxin, ML-9, MLN608, NSC- 30 226080, NSC-231634, NSC-664704, NSC-680410, NU6102, Olomoucine, Oxindole I, PD 153035, PD 98059, Phlorizdin, Piceatannol, Picropodophyllin, PKI, PP1, PP2, PTK787/ZK222584, PTK787/ZK-222584, Purvalanol A, Rapamune, Rapamycin, Ro 31-8220, Rottlerin, SB202190, 35 SB203580, Sirolimus, SL327, SP600125, Staurosporine, STI-571, SU1498, SU4312, SU5416, SU5416 (Semaxanib), SU6656, SU6668, syk inhibitor, TBB, TCN, Tyrophostin AG 1024, Tyrophostin AG 490, Tyrophostin AG 825, Tyrophostin AG 957, U0126, W-7, Wortmannin, Y-27632, Zactima 40 (ZD6474), ZM 252868, gefitinib (Iressa®), sunitinib malate (Sutent®; SU11248), erlotinib (Tarceva®; OSI-1774), lapatinib (GW572016; GW2016), canertinib (CI 1033), semaxanib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), imatinib (Gleevec®; ST1571), dasatinib 45 (BMS-354825), leflunomide (SU101), vandetanib (Zactima®; ZD6474), and nilotinib. Additional protein kinase inhibitors suitable for use in the present invention are described in, e.g., U.S. Pat. Nos. 5,618,829, 5,639,757, 5,728,868, 5,804,396, 6,100,254, 6,127,374, 6,245,759, 6,306,874, 6,313,138, 6,316,444, 6,329,380, 6,344,459, 6,420,382, 6,479,512, 6,498,165, 6,544,988, 6,562,818, 6,586,423, 6,586,424, 6,740,665, 6,794,393, 6,875,767, 6,927,293, and 6,958,340.

In some embodiments, the protein kinase inhibitor is a 55 multi-kinase inhibitor, which is an inhibitor that acts on more than one specific kinase. Examples of multi-kinase inhibitors include imatinib, sorafenib, lapatinib, BIRB-796, and AZD-1152, AMG706, Zactima (ZD6474), MP-412, sorafenib (BAY 43-9006), dasatinib, CEP-701 (lestaurtinib), XL647, XL999, Tykerb (lapatinib), MLN518, (formerly known as CT53518), PKC412, ST1571, AEE 788, OSI-930, 60 OSI-817, sunitinib malate (Sutent), axitinib (AG-013736), erlotinib, gefitinib, axitinib, bosutinib, temsirolimus and nilotinib (AMN107). In some particular embodiments, the tyrosine kinase inhibitor is sunitinib, sorafenib, or a pharmaceutically acceptable salt or derivative (such as a malate or a tosylate) of sunitinib or sorafenib.

33

Sunitinib malate, which is marketed by Pfizer Inc. under the trade name SUTENT, is described chemically as butanedioic acid, hydroxy-, (2S)-, compound with N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-1,2-dihydro-2-oxo-3H-indol-3-ylidine)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide (1:1). The compound, its synthesis, and particular polymorphs are described in U.S. Pat. No. 6,573,293, U.S. Patent Publication Nos. 2003-0229229, 2003-0069298 and 2005-0059824, and in J. M. Manley, M. J. Kalman, B. G. Conway, C. C. Ball, J. L. Havens and R. Vaidyanathan, "Early Amination Approach to 3-[(4-amido)pyrrol-2-yl]-2-indolones," *J. Org. Chew.* 68, 6447-6450 (2003). Formulations of sunitinib and its L-malate salt are described in PCT Publication No. WO 2004/024127. Sunitinib malate has been approved in the U.S. for the treatment of gastrointestinal stromal tumor, advanced renal cell carcinoma, and progressive, well-differentiated pancreatic neuroendocrine tumors in patients with unresectable locally advanced or metastatic disease. The recommended dose of sunitinib malate for gastrointestinal stromal tumor (GIST) and advanced renal cell carcinoma (RCC) for humans is 50 mg taken orally once daily, on a schedule of 4 weeks on treatment followed by 2 weeks off (Schedule 4/2). The recommended dose of sunitinib malate for pancreatic neuroendocrine tumors (pNET) is 37.5 mg taken orally once daily.

In the vaccine-based immunotherapy regimen, sunitinib malate may be administered orally in a single dose or multiple doses. Typically, sunitinib malate is delivered for two, three, four or more consecutive weekly doses followed by a "off" period of about 1 or 2 weeks, or more where no sunitinib malate is delivered. In one embodiment, the doses are delivered for about 4 weeks, with 2 weeks off. In another embodiment, the sunitinib malate is delivered for two weeks, with 1 week off. However, it may also be delivered without a "off" period for the entire treatment period. The effective amount of sunitinib malate administered orally to a human in the vaccine-based immunotherapy regimen is typically below 40 mg per person per dose. For example, it may be administered orally at 37.5, 31.25, 25, 18.75, 12.5, 6.25 mg per person per day. In some embodiments, sunitinib malate is administered orally in the range of 1-25 mg per person per dose. In some other embodiments, sunitinib malate is administered orally in the range of 6.25, 12.5, or 18.75 mg per person per dose. Other dosage regimens and variations are foreseeable, and will be determined through physician guidance.

Sorafenib tosylate, which is marketed under the trade name NEXAVAR, is also a multi-kinase inhibitor. Its chemical name is 4-(4-{3-[4-Chloro-3-(trifluoromethyl)phenyl]ureido}phenoxy)-N-methylpyrid-ine-2-carboxamide. It is approved in the U.S. for the treatment of primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer (hepatocellular carcinoma). The recommended daily dose is 400 mg taken orally twice daily. In the vaccine-based immunotherapy regimen provided by the present disclosure, the effective amount of sorafenib tosylate administered orally is typically below 400 mg per person per day. In some embodiments, the effective amount of sorafenib tosylate administered orally is in the range of 10-300 mg per person per day. In some other embodiments, the effective amount of sorafenib tosylate administered orally is between 10-200 mg per person per day, such as 10, 20, 60, 80, 100, 120, 140, 160, 180, or 200 mg per person per day.

Axitinib, which is marketed under the trade name INLYTA, is a selective inhibitor of VEGF receptors 1, 2, and 3. Its chemical name is (N-Methyl-2-[3-((E)-2-pyridin-2-yl-

34

vinyl)-1H-indazol-6-ylsulfanyl]-benzamide. It is approved for the treatment of advanced renal cell carcinoma after failure of one prior systemic therapy. The starting dose is 5 mg orally twice daily. Dose adjustments can be made based on individual safety and tolerability. In the vaccine-based immunotherapy regimen provided by the present disclosure, the effective amount of axitinib administered orally is typically below 5 mg twice daily. In some other embodiments, the effective amount of axitinib administered orally is between 1-5 mg twice daily. In some other embodiments, the effective amount of axitinib administered orally is between 1, 2, 3, 4, or 5 mg twice daily.

In the vaccine-based immunotherapy regimens any IEC enhancers may be used. They may be small molecules or large molecules (such as protein, polypeptide, DNA, RNA, and antibody). Examples of IEC enhancers that may be used include TNFR agonists, CTLA-4 antagonists, TLR agonists, programmed cell death protein 1 (PD-1) antagonists (such as BMS-936558), anti-PD-1 antibody CT-011), and programmed cell death protein 1 ligand 1 (PD-L1) antagonists (such as BMS-936559), lymphocyte-activation gene 3 (LAG3) antagonists, and T cell Immunoglobulin- and mucin-domain-containing molecule-3 (TIM-3) antagonists. Examples of specific TNFR agonists, CTLA-4 antagonists, and TLR agonists are provided in details herein below.

TNFR Agonists.

Examples of TNFR agonists include agonists of OX40, 4-1BB (such as BMS-663513), GITR (such as TRX518), and CD40. Examples of specific CD40 agonists are described in details herein below.

CD40 agonists are substances that bind to a CD40 receptor on a cell and is capable of increasing one or more CD40 or CD40L associated activities. Thus, CD40 "agonists" encompass CD40 "ligands".

Examples of CD40 agonists include CD40 agonistic antibodies, fragments CD40 agonistic antibodies, CD40 ligands (CD40L), and fragments and derivatives of CD40L such as oligomeric (e.g., bivalent, trimeric CD40L), fusion proteins containing and variants thereof.

CD40 ligands for use in the present invention include any peptide, polypeptide or protein, or a nucleic acid encoding a peptide, polypeptide or protein that can bind to and activate one or more CD40 receptors on a cell. Suitable CD40 ligands are described, for example, in U.S. Pat. Nos. 6,482,411, 6,410,711; U.S. Pat. No. 6,391,637; and U.S. Pat. No. 5,981,724, all of which patents and application and the CD40L sequences disclosed therein are incorporated by reference in their entirety herein. Although human CD40 ligands will be preferred for use in human therapy, CD40 ligands from any species may be used in the invention. For use in other animal species, such as in veterinary embodiments, a species of CD40 ligand matched to the animal being treated will be preferred. In certain embodiments, the CD40 ligand is a gp39 peptide or protein oligomer, including naturally forming gp39 peptide, polypeptide or protein oligomers, as well as gp39 peptides, polypeptides, proteins (and encoding nucleic acids) that comprise an oligomerization sequence. While oligomers such as dimers, trimers and tetramers are preferred in certain aspects of the invention, in other aspects of the invention larger oligomeric structures are contemplated for use, so long as the oligomeric structure retains the ability to bind to and activate one or more CD40 receptor(s).

In certain other embodiments, the CD40 agonist is an anti-CD40 antibody, or antigen-binding fragment thereof. The antibody can be a human, humanized or part-human chimeric anti-CD40 antibody. Examples of specific anti-

35

CD40 monoclonal antibodies include the G28-5, mAb89, EA-5 or S2C6 monoclonal antibody, and CP870893. In a particular embodiment, the anti-CD40 agonist antibody is CP870893 or dacetuzumab (SGN-40).

CP-870,893 is a fully human agonistic CD40 monoclonal antibody (mAb) that has been investigated clinically as an anti-tumor therapy. The structure and preparation of CP870,893 is disclosed in WO2003041070 (where the antibody is identified by the internal identifier “21.4.1”). The amino acid sequences of the heavy chain and light chain of CP-870,893 are set forth in SEQ ID NO: 40 and SEQ ID NO: 41, respectively. In clinical trials, CP870,893 was administered by intravenous infusion at doses generally in the ranges of 0.05-0.25 mg/kg per infusion. In a phase I clinical study, the maximum tolerated dose (MTD) of CP-870893 was estimated to be 0.2 mg/kg and the dose-limiting toxicities included grade 3 CRS and grade 3 urticaria. [Jens Ruter et al.: Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors. *Cancer Biology & Therapy* 10:10, 983-993; Nov. 15, 2010.]. In the vaccine-based immunotherapy regimen provided by the present disclosure, CP-870,893 can be administered intradermally, subcutaneously, or topically. It is preferred that it is administered intradermally. The effective amount of CP870893 to be administered in the regimen is generally below 0.2 mg/kg, typically in the range of 0.01 mg-0.15 mg/kg, or 0.05-0.1 mg/kg.

Dacetuzumab (also known as SGN-40 or huS2C6; CAS number 88-486-59-9) is another anti-CD40 agonist antibody that has been investigated in clinical trials for indolent lymphomas, diffuse large B cell lymphomas and Multiple Myeloma. In the clinical trials, dacetuzumab was administered intravenously at weekly doses ranging from 2 mg/kg to 16 mg/kg. In the vaccine-based immunotherapy regimen provided by the present disclosure, dacetuzumab can be administered intradermally, subcutaneously, or topically. It is preferred that it is administered intradermally. The effective amount of dacetuzumab to be administered in the vaccine-based immunotherapy regimen is generally below 16 mg/kg, typically in the range of 0.2 mg-14 mg/kg, or 0.5-8 mg/kg, or 1-5 mg/kg.

CTLA-4 Inhibitors.

Suitable anti-CTLA-4 antagonist agents for use in the vaccine-based immunotherapy regimen provided by the disclosure include, without limitation, anti-CTLA-4 antibodies (such as human anti-CTLA-4 antibodies, mouse anti-CTLA-4 antibodies, mammalian anti-CTLA-4 antibodies, humanized anti-CTLA-4 antibodies, monoclonal anti-CTLA-4 antibodies, polyclonal anti-CTLA-4 antibodies, chimeric anti-CTLA-4 antibodies, anti-CTLA-4 domain antibodies), fragments of anti-CTLA-4 antibodies (such as (single chain anti-CTLA-4 fragments, heavy chain anti-CTLA-4 fragments, and light chain anti-CTLA-4 fragments), and inhibitors of CTLA-4 that agonize the co-stimulatory pathway. In some embodiments, the CTLA-4 inhibitor is Ipilimumab or Tremelimumab.

Ipilimumab (also known as MEX-010 or MDX-101), marketed as YERVOY, is a human anti-human CTLA-4 antibody. Ipilimumab can also be referred to by its CAS Registry No. 477202-00-9, and is disclosed as antibody 10D1 in PCT Publication No. WO 01/14424, incorporated herein by reference in its entirety and for all purposes. Examples of pharmaceutical composition comprising Ipilimumab are provided in PCT Publication No. WO 2007/67959. Ipilimumab is approved in the U.S. for the treatment of unresectable or metastatic melanoma. The recommended dose of Ipilimumab as monotherapy is 3 mg/kg by intrave-

36

nous administration every 3 weeks for a total of 4 doses. In the methods provided by the present invention, Ipilimumab is administered locally, particularly intradermally or subcutaneously. The effective amount of Ipilimumab administered locally is typically in the range of 5-200 mg/dose per person. In some embodiments, the effective amount of Ipilimumab is in the range of 10-150 mg/dose per person per dose. In some particular embodiments, the effective amount of Ipilimumab is about 10, 25, 50, 75, 100, 125, 150, 175, or 200 mg/dose per person.

Tremelimumab (also known as CP-675,206) is a fully human IgG2 monoclonal antibody and has the CAS number 745013-59-6. Tremelimumab is disclosed as antibody 11.2.1 in U.S. Pat. No. 6,682,736, incorporated herein by reference in its entirety and for all purposes. The amino acid sequences of the heavy chain and light chain of Tremelimumab are set forth in SEQ IND NOS:42 and 43, respectively. Tremelimumab has been investigated in clinical trials for the treatment of various tumors, including melanoma and breast cancer; in which Tremelimumab was administered intravenously either as single dose or multiple doses every 4 or 12 weeks at the dose range of 0.01 and 15 mg/kg. In the regimens provided by the present invention, Tremelimumab is administered locally, particularly intradermally or subcutaneously. The effective amount of Tremelimumab administered intradermally or subcutaneously is typically in the range of 5-200 mg/dose per person. In some embodiments, the effective amount of Tremelimumab is in the range of 10-150 mg/dose per person per dose. In some particular embodiments, the effective amount of Tremelimumab is about 10, 25, 50, 75, 100, 125, 150, 175, or 200 mg/dose per person.

Toll-Like Receptor (TLR) Agonists.

The term “toll-like receptor agonist” or “TLR agonist” refers to a compound that acts as an agonist of a toll-like receptor (TLR). This includes agonists of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, and TLR11 or a combination thereof. Unless otherwise indicated, reference to a TLR agonist compound can include the compound in any pharmaceutically acceptable form, including any isomer (e.g., diastereomer or enantiomer), salt, solvate, polymorph, and the like. In particular, if a compound is optically active, reference to the compound can include each of the compound’s enantiomers as well as racemic mixtures of the enantiomers. Also, a compound may be identified as an agonist of one or more particular TLRs (e.g., a TLR7 agonist, a TLR8 agonist, or a TLR7/8 agonist).

The TLR agonism for a particular compound may be assessed in any suitable manner known in the art. Regardless of the particular assay employed, a compound can be identified as an agonist of a particular TLR if performing the assay with a compound results in at least a threshold increase of some biological activity mediated by the particular TLR. Conversely, a compound may be identified as not acting as an agonist of a specified TLR if, when used to perform an assay designed to detect biological activity mediated by the specified TLR, the compound fails to elicit a threshold increase in the biological activity. Unless otherwise indicated, an increase in biological activity refers to an increase in the same biological activity over that observed in an appropriate control. An assay may or may not be performed in conjunction with the appropriate control. With experience, one skilled in the art may develop sufficient familiarity with a particular assay (e.g., the range of values observed in an appropriate control under specific assay conditions) that performing a control may not always be necessary to determine the TLR agonism of a compound in a particular assay.

Certain TLR agonists useful in the method of the present invention are small organic molecules, as opposed to large biological molecules such as proteins, peptides, and the like. Examples of small molecule TLR agonists include those disclosed in, for example, U.S. Pat. Nos. 4,689,338; 4,929,624; 4,988,815; 5,037,986; 5,175,296; 5,238,944; 5,266,575; 5,268,376; 5,346,905; 5,352,784; 5,367,076; 5,389,640; 5,395,937; 5,446,153; 5,482,936; 5,693,811; 5,741,908; 5,756,747; 5,939,090; 6,039,969; 6,083,505; 6,110,929; 6,194,425; 6,245,776; 6,331,539; 6,376,669; 6,451,810; 6,525,064; 6,545,016; 6,545,017; 6,558,951; and 6,573,273. Examples of specific small molecule TLR agonists useful in the methods provided by the present invention include 4-amino-alpha, alpha,2-trimethyl-IH-imidazo[4,5-c]quolinol-I-ethanol, N-(2-[2-[4-amino-2-(2-methoxyethyl)-IH-imidazo[4,5-c]quinolin-I-yl]ethoxy]-ethyl)-N-methylmorpholine-4-carboxamide, I-(2-amino-2-methylpropyl)-2-(ethoxymethyl)-IH-imidazo[4,5-c]quinolin-4-arnine, N-[4-(4-amino-2-ethyl-IH-imidazo[4,5-c]quinolin-I-yl)butyl]methanesulfonamide, N-[4-(4-amino-2-propyl-IH-imidazo[4,5-c]quinolin-I-yl)butyl]methanesulfonamide, and imiquimod. Some TLR agonists particularly useful in the methods or regimen provided by the present disclosure are discussed in review article: Folkert Steinhagen, et al.: TLR-based immune adjuvants. Vaccine 29 (2011): 3341-3355.

In some embodiments, the TLR agonists are TLR9 agonists, particularly CpG oligonucleotides (or CpG.ODN). A CpG oligonucleotide is a short nucleic acid molecule containing a cytosine followed by a guanine linked by a phosphate bond in which the pyrimidine ring of the cytosine is unmethylated. A CpG motif is a pattern of bases that include an unmethylated central CpG surrounded by at least one base flanking (on the 3' and the 5' side of) the central CpG. CpG oligonucleotides include both D and K oligonucleotides. The entire CpG oligonucleotide can be unmethylated or portions may be unmethylated. Examples of CpG oligonucleotides useful in the methods provided by the present disclosure include those disclosed in U.S. Pat. Nos. 6,194,388, 6,207,646, 6,214,806, 628,371, 6,239,116, and 6,339,068.

The CpG oligonucleotides can encompass various chemical modifications and substitutions, in comparison to natural RNA and DNA, involving a phosphodiester internucleoside bridge, a beta-D-ribose (deoxyribose) unit and/or a natural nucleoside base (adenine, guanine, cytosine, thymine, uracil). Examples of chemical modifications are known to the skilled person and are described, for example in Uhlmann E. et al. (1990), Chem. Rev. 90:543; "Protocols for Oligonucleotides and Analogs", Synthesis and Properties and Synthesis and Analytical Techniques, S. Agrawal, Ed., Humana Press, Totowa, USA 1993; Crooke, S T. et al. (1996) Annu. Rev. Pharmacol. Toxicol. 36:107-129; and Hunziker J. et al., (1995), Mod. Synth. Methods 7:331-417. Specifically, a CpG oligonucleotide can contain a modified cytosine. A modified cytosine is a naturally occurring or non-naturally occurring pyrimidine base analog of cytosine which can replace this base without impairing the immunostimulatory activity of the oligonucleotide. Modified cytosines include but are not limited to 5-substituted cytosines (e.g. 5-methylcytosine, 5-fluorocytosine, 5-chloro-cytosine, 5-bromo-cytosine, 5-ido-cytosine, 5-hydroxy-cytosine, 5-hydroxymethyl-cytosine, 5-difluoromethyl-cytosine, and unsubstituted or substituted 5-alkynyl-cytosine), 6-substituted cytosines, N4-substituted cytosines (e.g. N4-ethyl-cytosine), 5-azacytosine, 2-mercaptop-cytosine, isocytosine, pseudo-isocytosine, cytosine analogs with condensed ring systems (e.g. N,N'-propylene cytosine or phenoxazine), and uracil and its

derivatives (e.g. 5-fluoro-uracil, 5-bromo-uracil, 5-bromovinyl-uracil, 4-thio-uracil, 5-hydroxy-uracil, 5-propynyl-uracil). Some of the preferred cytosines include 5-methylcytosine, 5-fluoro-cytosine, 5-hydroxy-cytosine, 5-hydroxymethyl-cytosine, and N4-ethyl-cytosine.

A CpG oligonucleotide can also contain a modified guanine. A modified guanine is a naturally occurring or non-naturally occurring purine base analog of guanine which can replace this base without impairing the immunostimulatory activity of the oligonucleotide. Modified guanines include but are not limited to 7-deazaguanine, 7-deaza-7-substituted guanine, hypoxanthine, N2-substituted guanines (e.g. N2-methyl-guanine), 5-amino-3-methyl-3H,6H-thiazolo[4,5-d]pyrimidine-2,7-dione, 2,6-diaminopurine, 2-aminopurine, purine, indole, adenine, substituted adenines (e.g. N6-methyl-adenine, 8-oxo-adenine), 8-substituted guanine (e.g. 8-hydroxyguanine and 8-bromoguanine), and 6-thioguanine. In some embodiments of the disclosure, the guanine base is substituted by a universal base (e.g. 4-methylindole, 5-nitro-indole, and K-base), an aromatic ring system (e.g. benzimidazole or dichloro-benzimidazole, 1-methyl-1H-[1,2,4]triazole-3-carboxylic acid amide) or a hydrogen atom.

In certain aspects, the CpG oligonucleotides include modified backbones. It has been demonstrated that modification of the nucleic acid backbone provides enhanced activity of nucleic acids when administered in vivo. Secondary structures, such as stem loops, can stabilize nucleic acids against degradation. Alternatively, nucleic acid stabilization can be accomplished via phosphate backbone modifications. A preferred stabilized nucleic acid has at least a partial phosphorothioate modified backbone. Phosphorothioates may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and alkyl-phosphonates can be made, e.g. as described in U.S. Pat. No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as described in U.S. Pat. No. 5,023,243 and European Patent No. 092,574) can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described (Uhlmann, E. and Peyman, A. (1990) Chem. Rev. 90:544; Goodchild, J. (1990) Bioconjugate Chem. 1:165). 2'-O-methyl nucleic acids with CpG motifs also cause immune activation, as do ethoxy-modified CpG nucleic acids. In fact, no backbone modifications have been found that completely abolish the CpG effect, although it is greatly reduced by replacing the C with a 5-methyl C. Constructs having phosphorothioate linkages provide maximal activity and protect the nucleic acid from degradation by intracellular exo- and endo-nucleases. Other modified oligonucleotides include phosphodiester modified oligonucleotides, combinations of phosphodiester and phosphorothioate oligonucleotides, methylphosphonate, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof. Each of these combinations and their particular effects on immune cells is discussed in more detail with respect to CpG nucleic acids in PCT Publication Nos. WO 96/02555 and WO 98/18810 and in U.S. Pat. Nos. 6,194,388 and 6,239,116.

The CpG oligonucleotides may have one or two accessible 5' ends. It is possible to create modified oligonucleotides having two such 5' ends, for instance, by attaching two oligonucleotides through a 3'-3' linkage to generate an oligonucleotide having one or two accessible 5' ends. The 3'-3'-linkage may be a phosphodiester, phosphorothioate or any other modified internucleoside bridge. Methods for

accomplishing such linkages are known in the art. For instance, such linkages have been described in Seliger, H. et al., Oligonucleotide analogs with terminal 3'-3' and 5'-5'-internucleotidic linkages as antisense inhibitors of viral gene expression, Nucleosides and Nucleotides (1991), 10(1-3), 469-77 and Jiang, et al., Pseudo-cyclic oligonucleotides: in vitro and in vivo properties, Bioorganic and Medicinal Chemistry (1999), 7(12), 2727-2735.

Additionally, 3'-3'-linked oligonucleotides where the linkage between the 3'-terminal nucleosides is not a phosphodiester, phosphorothioate or other modified bridge, can be prepared using an additional spacer, such as tri- or tetra-ethyleneglycol phosphate moiety (Durand, M. et al., Triple-helix formation by an oligonucleotide containing one (dA) 12 and two (dT)12 sequences bridged by two hexaethylene glycol chains, Biochemistry (1992), 31 (38), 9197-204, U.S. Pat. Nos. 5,658,738 and 5,668,265). Alternatively, the non-nucleotidic linker may be derived from ethanediol, propanediol, or from an abasic deoxybose (dSpacer) unit (Fontanel, Marie Laurence et al., Nucleic Acids Research (1994), 22(11), 2022-7) using standard phosphoramidite chemistry. The non-nucleotidic linkers can be incorporated once or multiple times, or combined with each other allowing for any desirable distance between the 3'-ends of the two oligonucleotides to be linked.

A phosphodiester internucleoside bridge located at the 3' and/or the 5' end of a nucleoside can be replaced by a modified internucleoside bridge, wherein the modified internucleoside bridge is for example selected from phosphorothioate, phosphorodithioate, NRiR₂-phosphoramidate, boronophosphate, a-hydroxybenzyl phosphonate, phosphate-(C₁-C₂₁)-O-alkyl ester, phosphate-[(C₆-C₂₁)aryl-(C₁-C₂₁)-O-alkyl]ester, (C₁-C₈)alkylphosphonate and/or (C₆-C₁₂)arylphosphonate bridges, (C₇-C₁₂)-a-hydroxymethyl-aryl (e.g. disclosed in PCT Publication No. WO 95/01363), wherein (C₆-C₁₂)aryl, (C₆-C₂₀)aryl and (C₆-C₁₄) aryl are optionally substituted by halogen, alkyl, alkoxy, nitro, cyano, and where R₁ and R₂ are, independently of each other, hydrogen, (C₁-C₁₈)-alkyl, (C₆-C₂₀)-aryl, (C₆-C₁₄)-aryl, (C₁-C₈)-alkyl, preferably hydrogen, (C₁-C₈)-alkyl, preferably (C₁-C₄)-alkyl and/or methoxyethyl, or R₁ and R₂ form, together with the nitrogen atom carrying them, a 5 to 6-membered heterocyclic ring which can additionally contain a further heteroatom selected from the group O, S and N.

The replacement of a phosphodiester bridge located at the 3' and/or the 5' end of a nucleoside by a dephospho bridge (dephospho bridges are described, for example, in Uhlmann E. and Peyman A. in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonucleotides and Analogs", S. Agrawal, Ed., Humana Press, Totowa 1993, Chapter 16, pp. 355 if), wherein a dephospho bridge is for example selected from the dephospho bridges formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethyl-hydrazone, dimethylenesulfone and/or silyl groups.

The CpG oligonucleotides for use in the methods or regimen provided by the disclosure may optionally have chimeric backbones. A chimeric backbone is one that comprises more than one type of linkage. In one embodiment, the chimeric backbone can be represented by the formula: 5' Y₁ N1ZN2Y₂ 3'. Y₁ and Y₂ are nucleic acid molecules having between 1 and 10 nucleotides. Y₁ and Y₂ each include at least one modified internucleotide linkage. Since at least 2 nucleotides of the chimeric oligonucleotides include backbone modifications these nucleic acids are an example of one type of "stabilized immunostimulatory nucleic acids".

With respect to the chimeric oligonucleotides, Y₁ and Y₂ are considered independent of one another. This means that each of Y₁ and Y₂ may or may not have different sequences and different backbone linkages from one another in the same molecule. In some embodiments, Y₁ and/or Y₂ have between 3 and 8 nucleotides. N1 and N2 are nucleic acid molecules having between 0 and 5 nucleotides as long as N1ZN2 has at least 6 nucleotides in total. The nucleotides of N1ZN2 have a phosphodiester backbone and do not include nucleic acids having a modified backbone. Z is an immunostimulatory nucleic acid motif, preferably selected from those recited herein.

The center nucleotides (N1ZN2) of the formula Y₁ N1ZN2Y₂ have phosphodiester internucleotide linkages and Y₁ and Y₂ have at least one, but may have more than one or even may have all modified internucleotide linkages. In preferred embodiments, Y₁ and/or Y₂ have at least two or between two and five modified internucleotide linkages or Y₁ has five modified internucleotide linkages and Y₂ has two modified internucleotide linkages. The modified internucleotide linkage, in some embodiments, is a phosphorothioate modified linkage, a phosphorodithioate linkage or a p-ethoxy modified linkage.

Examples of particular CpG oligonucleotides useful in the methods provided by the present disclosure include:

5' TCGTCGTTTGTGCGTTTGTCGTT3'; (CpG 7909)

5' TCGTCGTTTTCGGTGCTTT3'; (CpG 24555)
and

5' TCGTCGTTTTCGGTCGTTT3'. (CpG 10103)

CpG7909, a synthetic 24mer single stranded, has been extensively investigated for the treatment of cancer as a monotherapy and in combination with chemotherapeutic agents, as well as adjuvant as an adjuvant for vaccines against cancer and infectious diseases. It was reported that a single intravenous dose of CpG 7909 was well tolerated with no clinical effects and no significant toxicity up to 1.05 mg/kg, while a single dose subcutaneous CpG 7909 had a maximum tolerated dose (MTD) of 0.45 mg/kg with dose limiting toxicity of myalgia and constitutional effects. [See Zent, Clive S. et al: Phase I clinical trial of CpG oligonucleotide 7909 (PF-03512676) in patients with previously treated chronic lymphocytic leukemia. Leukemia and Lymphoma, 53(2):211-217(7)(2012)]. In the regimens provided by the present disclosure, CpG7909 may be administered by injection into the muscle or any other suitable methods. It is preferred that it is administered locally in proximity to the vaccine draining lymph node, particularly by intradermal or subcutaneous administration. For use with a nucleic acid vaccine, such as a DNA vaccine, a CpG may be preferably co-formulated with the vaccine in a single formulation and administered by intramuscular injection coupled with electroporation. The effective amount of CpG7909 by intramuscular, intradermal, or subcutaneous administration is typically in the range of 10 µg/dose-10 mg/dose. In some embodiments, the effective amount of CpG7909 is in the range of 0.05 mg-14 mg/dose. In some particular embodiments, the effective amount of CpG7909 is about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.5 1 mg/dose. Other CpG oligonucleotides, including CpG 24555 and CpG 10103, may be administered in similar manner and dose levels.

In some particular embodiments, the present disclosure provides a method of enhancing the immunogenicity or therapeutic effect of a vaccine for the treatment of a neo-

plastic disorder in a human, comprising administering the human (1) an effective amount of at least one ISC inhibitor and (2) an effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is protein kinase inhibitor selected from sorafenib tosylate, sunitinib malate, axitinib, erlotinib, gefitinib, axitinib, bosutinib, temsirolimus, or nilotinib and wherein the at least one IEC enhancer is selected from a CTLA-4 inhibitor, a TLR agonist, or a CD40 agonist. In some preferred embodiments, regimen comprises administering to the human (1) an effective amount of at least one ISC inhibitor and (2) effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from axitinib, sorafenib tosylate, or sunitinib malate and wherein the wherein the at least one IEC enhancer is a CTLA-4 inhibitor selected from Ipilimumab or Tremelimumab. In some further preferred embodiments, the regimen comprises administering to the human (1) an effective amount of at least one ISC inhibitor and (2) an effective amount of at least two IEC enhancers, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sunitinib or axitinib and wherein the at least two IEC enhancers are Tremelimumab and a TLR agonist selected from CpG7909, CpG2455, or CpG 10103.

In some other embodiments, the present disclosure provides a method of treating prostate cancer in a human, comprising administering to the human (1) an effective amount of a vaccine capable of eliciting an immune response against a human PAA, (2) an effective amount of at least one ISC inhibitor, and (3) an effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sorafenib tosylate, sunitinib malate, axitinib, erlotinib, gefitinib, axitinib, bosutinib, temsirolimus, or nilotinib, and wherein the at least one IEC enhancer is selected from a CTLA-4 inhibitor, a TLR agonist, or a CD40 agonist. In some preferred embodiments, the method comprises administering to the human (1) an effective amount of a vaccine capable of eliciting an immune response against a human PAA, (2) an effective amount of at least one ISC inhibitor, and (3) an effective amount of at least one IEC enhancer, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sorafenib tosylate, sunitinib malate, or axitinib and wherein the at least one IEC enhancer is a CTLA-4 inhibitor selected from Ipilimumab or Tremelimumab.

In some further specific embodiments, the method comprises administering to the human (1) an effective amount of at least one ISC inhibitor and (2) an effective amount of at least two IEC enhancers, wherein the at least one ISC inhibitor is a protein kinase inhibitor selected from sunitinib or axitinib and wherein the at least two IEC enhancers are Tremelimumab and a TLR agonist selected from CpG7909, CpG2455, or CpG10103.

Additional Therapeutic Agents.

The vaccine-based immunotherapy regimen provided by the present disclosure may further comprise an additional therapeutic agent. A wide variety of cancer therapeutic agents may be used, including chemotherapeutic agents and hormone therapeutic agents. One of ordinary skill in the art will recognize the presence and development of other cancer therapies which can be used in VBIR provided by the present disclosure, and will not be restricted to those forms of therapy set forth herein.

The term "chemotherapeutic agent" refers to a chemical or biological substance that can cause death of cancer cells, or interfere with growth, division, repair, and/or function of

cancer cells. Examples of chemotherapeutic agents include those that are disclosed in WO2006/088639, WO2006/129163, and US 20060153808, the disclosures of which are incorporated herein by reference. Examples of particular chemotherapeutic agents include: (1) alkylating agents, such as chlorambucil (LEUKERAN), cyclophosphamide (CYTOXAN), ifosfamide (IFEX), mechlorethamine hydrochloride (MUSTARGEN), thiotapec (THIOPLEX), streptozotocin (ZANOSAR), carmustine (BICNU, GLIADEL WAFER), lomustine (CEENU), and dacarbazine (DTIC-DOME); (2) alkaloids or plant vinca alkaloids, including cytotoxic antibiotics, such as doxorubicin (ADRIAMYCIN), epirubicin (ELLENCE, PHARMORUBICIN), daunorubicin (CERUBIDINE, DAUNOXOME), nemorubicin, idarubicin (IDAMYCIN PFS, ZAVEDOS), mitoxantrone (DHAD, NOVANTRONE). dactinomycin (actinomycin D, COSMEGEN), plicamycin (MITHRACIN), mitomycin (MUTAMYCIN), and bleomycin (BLENOXANE), vinorelbine tartrate (NAVELBINE), vinblastine (VELBAN), vincristine (ONCOVIN), and vindesine (ELDISINE); (3) antimetabolites, such as capecitabine (XELODA), cytarabine (CYTOSAR-U), fludarabine (FLUDARA), gemcitabine (GEMZAR), hydroxyurea (HYDRA), methotrexate (FOLEX, MEXATE, TREXALL), nelarabine (ARRANON), trimetrexate (NEUTREXIN), and pemetrexed (ALIMTA); (4) Pyrimidine antagonists, such as 5-fluorouracil (5-FU); capecitabine (XELODA), raltitrexed (TOMUDEX), tegafur-uracil (UFTORAL), and gemcitabine (GEMZAR); (5) taxanes, such as docetaxel (TAXOTERE), paclitaxel (TAXOL); (6) platinum drugs, such as cisplatin (PLATINOL) and carboplatin (PARAPLATIN), and oxaliplatin (ELOXATIN); (7) topoisomerase inhibitors, such as irinotecan (CAMPTOSAR), topotecan (HYCAMTIN), etoposide (ETOPOPHOS, VEPESSID, TOPOSAR), and teniposide (VUMON); (8) epipodophyllotoxins (podophyllotoxin derivatives), such as etoposide (ETOPOPHOS, VEPESSID, TOPOSAR); (9) folic acid derivatives, such as leucovorin (WELLCOVORIN); (10) nitrosoureas, such as carmustine (BICNU), lomustine (CeeNU); (11) inhibitors of receptor tyrosine kinase, including epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), insulin receptor, insulin-like growth factor receptor (IGFR), hepatocyte growth factor receptor (HGFR), and platelet-derived growth factor receptor (PDGFR), such as gefitinib (IRESSA), erlotinib (TARCEVA), bortezomib (VELCADE), imatinib mesylate (GLEEVEC), genfitinib, lapatinib, sorafenib, thalidomide, sunitinib (SUTENT), axitinib, rituximab (RITUXAN, MABTHERA), trastuzumab (HERCEPTIN), cetuximab (ERBITUX), bevacizumab (AVASTIN), and ranibizumab (LUCENTIS), lym-1 (ONCOLYM), antibodies to insulin-like growth factor-1 receptor (IGF-1R) that are disclosed in WO2002/053596; (12) angiogenesis inhibitors, such as bevacizumab (AVASTIN), suramin (GERMANIN), angiostatin, SU5416, thalidomide, and matrix metalloproteinase inhibitors (such as batimastat and marimastat), and those that are disclosed in WO2002055106; and (13) proteasome inhibitors, such as bortezomib (VELCADE).

The term "hormone therapeutic agent" refers to a chemical or biological substance that inhibits or eliminates the production of a hormone, or inhibits or counteracts the effect of a hormone on the growth and/or survival of cancer cells. Examples of such agents suitable for the VBIR include those disclosed in US20070117809. Examples of particular hormone therapeutic agents include tamoxifen (NOLVADEX), toremifene (Fareston), fulvestrant (FASLODEX), anastrozole (ARIMIDEX), exemestane (AROMASIN), letrozole

(FEMARA), megestrol acetate (MEGACE), goserelin (ZOLDEX), leuprolide (LUPRON), abiraterone, and MDV3100.

The VBIR provided by this disclosure may also be used in combination with other therapies, including (1) surgical methods that remove all or part of the organs or glands which participate in the production of the hormone, such as the ovaries, the testicles, the adrenal gland, and the pituitary gland, and (2) radiation treatment, in which the organs or glands of the patient are subjected to radiation in an amount sufficient to inhibit or eliminate the production of the targeted hormone.

I. EXAMPLES

The following examples are provided to illustrate certain embodiments of the invention. They should not be construed to limit the scope of the invention in any way. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of the invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usage and conditions.

Example 1

Antigens in Cytosolic, Secreted, and Membrane-Bound Formats Derived from the Human PSMA Protein

Example 1 illustrates the construction of three immunogenic PSMA polypeptides referred to as "human PSMA cytosolic antigen," "human PSMA secreted antigen," and "human PSMA membrane-bound antigen," respectively, and biological properties of these polypeptides.

1A. Design of Immunogenic PSMA Polypeptides

DNA constructs encoding immunogenic PSMA polypeptides in cytosolic, secreted, and modified formats were constructed based on the native human PSMA protein sequence and tested for their ability to induce anti-tumor effector immune responses. The structure and preparation of each of the human PSMA antigen formats are provided as follows.

1A1. Human PSMA Cytosolic Antigen.

An immunogenic PSMA polypeptide in cytosolic form was designed to retain the immunogenic polypeptide inside the cell once it is expressed. The cytoplasmic domain (amino acids 1-19) and the transmembrane domain (amino acids 20-43) of the human PSMA were removed, resulting in a cytosolic PSMA polypeptide that consists of amino acids 44-750 (extracellular domain or ECD) of the human PSMA of SEQ ID NO: 1. The optimal Kozak sequence "MAS" may be added to the N-terminus of the polypeptide for enhancing the expression.

1A2. Human PSMA Secreted Antigen.

An immunogenic PSMA polypeptide in secreted form was designed to secrete the polypeptide outside of the cell once it is expressed. The secreted polypeptide is made with amino acids 44-750 (ECD) of the human PSMA of SEQ ID NO: 1 and the Ig Kappa secretory element that has the amino acid sequence ETDTLLWVLLWVPGSTGD and a two-amino acid linker (AA) in the N-terminal in order to maximize the secretion of the PSMA antigen once it is expressed.

1A3. Human PSMA Membrane-Bound Antigen.

An immunogenic PSMA membrane-bound polypeptide was designed to stabilize the polypeptide on the cell surface.

The first 14 amino acids of the human PSMA protein were removed and the resultant immunogenic polypeptide consists of amino acids 15-750 of the human PSMA protein of SEQ ID NO: 1. The immunogenic polypeptide that consists of amino acids 15-750 of the native human PSMA protein of SEQ ID NO: 1 and share 100% sequence identity with the native human PSMA protein is also referred to as "human PSMA modified," "hPSMA modified," or "hPSMAmod" antigen in the present disclosure.

1B. Preparation of DNA Plasmids for Expressing the PSMA Antigens

DNA constructs encoding the PSMA cytosolic, PSMA secreted, and PSMA modified antigens were cloned individually into PJV7563 vector that was suitable for in vivo testing in animals (FIG. 1). Both strands of the DNA in the PJV7563 vectors were sequenced to confirm the design integrity.

A large scale plasmid DNA preparation (Qiagen/CsCl) was produced from a sequence confirmed clone. The quality of the plasmid DNA was confirmed by high 260/280 ratio, high super coiled/nicked DNA ratio, low endotoxin levels (<10 U/mg DNA) and negative bio burden.

1C. Expression of PSMA Constructs in Mammalian Cells

The expression of the PSMA cytosolic, secreted, and modified antigens was determined by FACS. Mammalian 293 cells were transfected with the PJV7563 PMED vectors encoding the various immunogenic PSMA polypeptides. Three days later, the 293 cells were stained with mouse anti-PSMA antibody, followed with a fluorescent conjugated (FITC) rat anti-mouse secondary antibody. The data below, which were reported as mean fluorescent intensity (MFI) over negative controls, confirmed that human PSMA modified antigen is expressed on the cell surface.

Samples	Average mean fluorescent intensity
Untransfected 293 cells	231
293 cells transfected with full length human PSMA (SEQ ID NO: 1)	6425
293 cells transfected with human PSMA modified antigen (SEQ ID NO: 9)	12270

1D. Formulations of PSMA Plasmids onto Gold Particles (for ND10/X15)

Particle Mediated Epidermal Delivery technology (PMED) is a needle-free method of administering vaccines to animals or to patients. The PMED system involves the precipitation of DNA onto microscopic gold particles that are then propelled by helium gas into the epidermis. The ND10, a single use device, uses pressurized helium from an internal cylinder to deliver gold particles and the $\times 15$, a repeater delivery device, uses an external helium tank which is connected to the $\times 15$ via high pressure hose to deliver the gold particles. Both of these devices were used in studies to deliver the PSMA DNA plasmids. The gold particle was usually 1-3 μm in diameter and the particles were formulated

to contain 2 μg of PSMA plasmids per 1 mg of gold particles. (Sharpe, M. et al.: P. Protection of mice from H5N1 influenza challenge by prophylactic DNA vaccination using particle mediated epidermal delivery. Vaccine, 2007, 25(34): 6392-98; Roberts L K, et al.: Clinical safety and efficacy of a powdered Hepatitis B nucleic acid vaccine delivered to the epidermis by a commercial prototype device. Vaccine, 2005; 23(40):4867-78).

1E. Transgenic Mice Used for In Vivo Studies

Two human HLA transgenic mouse models were used to evaluate the presentation of various PSMA antigens by different HLAs and a human PSMA transgenic mouse model was used to assess the breaking of immune tolerance to human PSMA. The first HLA transgenic mouse model utilizes the HLA A2/DR1 mice (from the Pasteur Institute, Paris, France; also referred to as "Pasteur mice"). Pasteur mice are knock out for murine β -2-microglobulin and do not express functional H-2b molecules; therefore this model is believed to represent the presentation of antigen in the human HLA A2 and DR1 context (Pajot, A., M.-L. Michel, N. Faxilleau, V. Pancre, C. Auriault, D. M. Ojcius, F. A. Lemonnier, and Y.-C. Lone. A mouse model of human adaptive immune functions: HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout mice. Eur. J. Immunol. 2004, 34:3060-69). The second HLA transgenic mouse model uses mice that are knock in with human HLA A24 that is covalently linked to the human β -2-microglobulin at the H2bk locus. These mice lack murine β -2-microglobulin and do not express functional H-2b molecules. This model allows evaluation of antigen presentation in the context of human HLA A24.

1F. Immunogenicity of the Human PSMA Proteins in Cytosolic, Secreted and Modified Formats

Study Design.

Eight-to-10 week-old transgenic mice were immunized using PMED method with various PSMA DNA constructs in a prime/boost/boost regimen, two weeks apart between each vaccination. Alternatively, mice were primed with adenovirus vectors encoding the PSMA antigen at 1×10^9 viral particles in 50 μ l (PBS) by intramuscular injection. The adenovirus vector (pShuttle-CMV vector from Stratagene) was modified to contain NheI and BglII restriction sites within the multiple cloning site. The DNA encoding human PSMA modified was then restriction digested with NheI and BglII, ligated into this vector and sequence confirmed. The pShuttle human PSMA modified vector was then recombined with the pAdEasy-1 vector and virus was propagated according to the AdEasy system (Stratagene). Twenty-days later, they were boosted with PMED as described above. In each of the regimens used, antigen specific T cell response was measured 7 days after the last immunization in an interferon-gamma (IFN γ) ELISPOT assay. The ELISPOT assay is similar to the sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, a capture antibody specific to IFN γ BD Bioscience, #51-2525Kc) is coated onto a polyvinylidene fluoride (PVDF) membrane in a microplate overnight at 4° C. The plate is blocked with serum/protein to prevent nonspecific binding to the antibody. After blocking, effector cells (such as splenocytes isolated from PSMA immunized mice) and targets (such as PSMA peptides from peptide library, target cells pulsed with peptides or tumor cells expressing the relevant antigens) or mitogen (which will stimulate splenocytes non-specifically to produce IFN γ) are added to the wells and incubated overnight at 37° C. in a 5% CO₂ incubator. Cytokine secreted by effector cells are captured by the coating antibody on the surface of the PVDF membrane. After removing the cells and culture media, 100 μ l of a biotinylated polyclonal anti-mouse IFN γ antibody (0.5 mg/ml-BD Bioscience, #51-1818Kz) was added to each of the wells for detection. The spots are visualized by adding streptavidin-horseradish peroxidase (HRP, BD Bioscience, #557630) and the precipitate substrate, 3-amino-9-ethylcarbazole (AEC), to yield a red color spot. Each spot represents a single cytokine producing T cell. In general, in the studies disclosed here the ELISPOT assay was set up as follows:

5×10^5 splenocytes from PSMA immunized mice were cultured (1) in the presence of PSMA specific peptides derived from a PSMA peptide library (see Table 16) made of 15-amino acid peptides overlapping by 11 amino acids, (2) with known HLA A2.1 restricted PSMA specific peptides, or (3) with tumor cells. To measure the recognition of endogenous antigen presentation, splenocytes were cultured with a human HLA A2 prostate cancer cells (i.e. LNCaP, available from ATCC) that naturally express PSMA or cultured with HLA A2 tumor cells transduced with adenovirus encoding and thus expressing the human PSMA modified antigen. In addition, human PSMA ECD protein was added to the ELISpot assay to measure specifically CD4 IFN γ producing cells. For controls where appropriate, HLA A2 restricted HER-2 specific peptide p168-175 or tumor cells not expressing PSMA or irrelevant protein such as BSA were used as a negative control in the IFN γ ELISpot assay. Data results are given in normalized format for the number of spot forming cells (SFC) that secrete IFN γ in 1×10^6 splenocytes. At least three studies were performed for each of the PSMA antigen peptides tested.

Results.

Data from the ELISpot assay with splenocytes of Pasteur mice cultured with peptides derived from a PSMA peptide library are presented in Table 1. A positive response is defined as having SFC >100. As shown in Table 1, the immunogenic PSMA polypeptides made with all three antigen formats, the human PSMA cytosolic, secreted, and modified antigens described in Example 1A above, are capable of inducing T cell responses. The human PSMA modified antigen format induced the best breadth and magnitude of T cell responses.

TABLE 1

T cell response induced by the human PSMA cytosolic, secreted, and modified antigens in Pasteur mice				
aa sequence coverage of target peptide pools from PSMA peptide library	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)			
	PSMA cytosolic	PSMA modified	PSMA secreted	
45	13-35	1(14)	809(78)	44(6)
	97-117	1(1)	96(17)	0
	109-131	8(9)	923(21)	179(24)
	121-243	21(4)	1329(109)	320(28)
	145-167	23(4)	1499(1)	312(14)
	169-191	497(4)	248(14)	183(13)
	181-203	43(21)	70(20)	19(10)
	205-227	5(1)	112(0)	9(13)
	217-239	44(13)	1627(38)	351(10)
	265-287	23(4)	527(100)	10(6)
	277-299	39(1)	1143(86)	151(4)
	289-311	27(1)	429(4)	28(11)
	409-431	14(9)	281(18)	12(9)
	421-443	4(0)	676(45)	48(14)
	433-455	339(18)	713(64)	119(21)
	481-503	22	288(9)	1(1)
	577-599	227(27)	131(16)	33(16)
	589-611	187(13)	27(10)	6(9)
	613-635	418(6)	437(1)	55(1)
	637-659	222(31)	49(10)	95(16)
50	649-671	203(21)	1625(33)	420(11)
	661-683	102(14)	1633(140)	366(48)
	697-719	179(4)	1357(58)	342(6)
	709-731	40(11)	1162(59)	223(4)
	721-743	56(6)	1409(103)	344(23)
	733-750	50(11)	1512(51)	365(27)

() = standard deviation

Data from the ELISpot assay on T cell responses induced by various PSMA vaccine formats in Pasteur mice (which that recognized HLA A2.1 restricted PSMA peptide pulsed target cells as well as PSMA+HLA A2.1 LNCaP tumor cells) are presented in Table 2. PC3, which is a human prostate cancer cell line that does not express PSMA, was used here as a negative control. A positive response is defined as having SFC >50. As shown in Table 2, the various PSMA constructs tested are capable of inducing T cells that recognize known HLA A2 restricted PSMA epitopes as well as PSMA protein and human prostate cancer cells LNCaP. However, the PSMA modified construct was shown to induce the best breadth and magnitude T cell response.

TABLE 2

T cell responses induced by the human PSMA cytosolic, secreted, and modified antigens in Pasteur mice that recognized HLA A2.1 restricted PSMA peptide pulsed target cells as well as PSMA+ HLA A2.1 LNCaP tumor cells.			
HLA A2.1 restricted peptides	PSMA cytosolic	PSMA modified	PSMA secreted
Target peptide or protein	IFN-γ SFC/1 × 10 ⁶ splenocytes		
PSMA p663	1554(4)	1524.9(45)	444(23)
PSMA p275	14(10)	304(21)	3(1)
PSMA p662	41(15)	925.1(77)	455(25)
PSMA p627	1(1)	222(14)	9(8)
PSA p64	0	2(4)	1(1)
Protein or tumor cells	IFN-γ SFC/1 × 10 ⁶ splenocytes		
PSMA ECD protein	45(11)	731(16)	13(5)
LNCap	4(2)	96(12)	5(3)
PC3	0	1(1)	1(1)

() = standard deviation

1G. Humoral Immune Response Measured in Pasteur Mice or Nonhuman Primates

1G1. Sandwich ELISA Assay.

The standard sandwich ELISA assay was done using an automated Biotek system. The plates were coated with 25 µl of native PSMA protein at a 1.0 µg/ml in PBS overnight, the plates were washed and blocked with 35 µl/well of 5% FBS 1×PBS-T 0.05% and incubated for 1 hour at RT on a shaker at 600 RPM. The blocking media was decanted and serial dilute vaccinated mouse serum with half log dilutions in 5% FBS 1×PBS-T 0.05% starting at 1:100 or 1:500 were made and 25µ samples of the diluted serum were added to each well of the 96 well plates and incubated for 1 hour at RT on a shaker at 600 RPM. The plates were washed 3 times with 75 ul/well in 1×PBS-T 0.05% using the Biotek ELx405, and 25 µl/well of 1:30,000 diluted anti-mouse IgG HRP (AbCam cat# ab20043) secondary antibody (diluted in 1× PBS-T 0.05%) was added to each well of the 96 well plates and incubated for 1 hour at RT on a shaker at 600 RPM. Plates were washed 5× with 75 ul/well in 1×PBS-T 0.05% using the Biotek Elx405. TMB Substrate was diluted at 1:10 and 25 µl was added to each well and incubated at RT for 30 minutes. The reaction was stopped by adding 12.5 µl/well of 1M H2SO4. Plates were read using the Spectramax Plus at 450 nm wavelength. Data were reported as titers and these could be reported as first positive (average and both values above 5% FBS PBS+3 time Standard Deviation) and/or as calculated titers at OD of 0.5 or 1.0. Serum from irrelevant vaccinated mice were used as negative controls.

TABLE 3

Induction of anti-PSMA antibody response by human PSMA antigens as measured by an ELISA assay.			
Antigen format	ELISA (OD = 1) Average (+/-SD)	N	# of positive
PSMA cytosolic	499 (0)	4	0/4
PSMA modified	1067 (518)	4	4/4
PSMA secreted	959 (920)	4	1/4

Results.

Data presented in Table 3 shows that the human PSMA cytosolic antigen did not induce any anti-PSMA responses, while the human PSMA modified antigen consistently induced good anti-PSMA antibody responses in all mice.

Data presented in Table 5 shows that antibodies induced by the human PSMA antigens reacted to multiple peptide epitopes in the PSMA library. Serum from the individual mice in each group was pooled in equal amounts and tested at a 1:500 dilution in an ELISA assay. A negative control group of mice vaccinated with anti-diphtheria (CRM) toxoid was tested in parallel. Each well of the 96 well ELISA plate was coated with 0.03 µg of a single 15aa peptide derived from the PSMA peptide library. An OD value above 0.10 is considered positive.

1G2. FACS Cell Binding Assay.

Various prostate cancer cell lines were used for this assay. LNCaP (ATCC) was used as human prostate cancer cells expressing PSMA and PC3 (ATCC) was used as negative human prostate cancer cells that do not express PSMA. In some assays, a TRAMP-C2 cell line engineered to stably express the human native full length PSMA and the parental TRAMP-C2 cell line that does not express PSMA (negative control) were used for the cell binding assay. The cell binding assay was performed as follows: LNCaP and PC3 cells (or TRAMP-C2PSMA and TRAMP C2) were plated in separate wells at 2×10⁵ cells/well (50 µL) in a 96 well plate. Sera from PSMA vaccinated mice, as described in 1f, were

diluted 1:50 with FACS buffer (PBS pH 7.4, 1% FBS, 25 mM HEPES, and 1 mM EDTA). Fifty µL of diluted J591-A antibody (mouse anti-human PSMA antibody, clone J591-A from ATCC) were added to the diluted test sera or FACS buffer (unstained samples) to achieve the appropriate cell numbers per well in the staining plate. All was mixed by pipetting and then kept on ice for 20 min. The cells were washed twice with FACS buffer; each wash was by centrifugation at 1200RPM at 5° C. for 5 minutes. Fifty µL of secondary staining solution were added containing a 1:200 dilution of PE-labeled goat anti-mouse Ig (Sigma, cat P9670-5) and 0.25 µL of Live/Dead Aqua stain (Invitrogen, cat. # L34957) to each of the cell containing wells and kept on ice for 20 min. Cells were washed twice as described earlier. Washed cell pellets were resuspended in 125 µL

FACS buffer and then 75 µL 4% paraformaldehyde solution were added to each well to fix the cells. Samples were kept on ice and protected from light for at least 15 min. Samples were run on FACS Canto II. Ten thousand live cell events were recorded for each sample. Control samples for each cell type were 1) unstained cells, 2) cells with secondary antibody only, 3) Cells with J591 plus secondary antibody, and 4) cells with naïve serum plus secondary antibody. Data were reported as mean fluorescent intensity (MFI) over negative controls.

Results of FACS Cell Binding Assay.

Table 4 shows that antibodies induced by both human PSMA secreted and modified antigens are capable of bind-

ing to human PSMA positive prostate cancer cells (LNCaP) and not to PSMA negative prostate cancer cells (PC3). The PSMA modified antigen consistently induced good anti-PSMA antibody response in all mice.

TABLE 4

Binding of anti-PSMA antibodies to human prostate cancer cells as measured by FACS.

Antigen Format	Fold over background Average (+/-SD)	N	# of positive
PSMA cytosolic	1.40 (0.12)	4	0/4
PSMA modified	6.01 (0.38)	4	4/4
PSMA secreted	5.50 (4.10)	4	3/4
background (PC3)	1.36 (0.04)	4	NA

TABLE 5

Antibodies induced by PSMA vaccine reacted to multiple peptides in the PSMA library. Based on this result, four B cell epitopes of PSMA were identified, 1: aa 138-147, 2: aa 119-123, 3: aa 103-106, 4: aa 649-659.

Target peptide or protein	ELISA O.D. results	
	PSMA vaccinated serum	CRM-197 vaccinated serum
Peptides from PSMA	0.21	0.05
library (no. of first aa)	0.27	0.05
133	0.58	0.05
137	0.89	0.05
141	0.12	0.05
645	0.17	0.05
PSMA protein	3.87	0.05

1 G3. Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Assay

Study Design.

An Indian rhesus macaque was immunized with a nucleic acid encoding a human PSMA modified antigen delivered by adenovirus (1e11 V.P. injected intramuscularly) followed by 2 PMED immunizations (8 actuations/immunization, 4 actuations per each right and left side of the lower abdomen) with 8 and 6 week intervals respectively. The animal also received intradermal injections of 3 mg of CpG (PF-03512676) in proximity to each inguinal draining lymph node at the time of the second PMED immunization. The antibody dependent cell-mediated cytotoxicity was determined from the plasma collected from the blood before any immunizations (pre-immune plasma) and 8 days after the last PMED immunization (immune plasma).

Antibody-Dependent Cell-Mediated Cytotoxicity Assay.

Antibody-dependent cell-mediated cytotoxicity was determined using the standard chromium 51 release assay. Human prostate cancer cell lines LNCaP and PC3 were used as target cells. Freshly isolated human PBMC cells were used as effector cells. Effectors to target cells were set at 30:1. Briefly, for one labeling reaction, 1.5×10^6 target cells in 200 μ l were incubated with 200 μ Ci ^{51}Cr (37°C , 5% CO_2 for 1 hour). Cells were washed three times and the cell concentration was adjusted to 2×10^5 cells/ml. Control monoclonal antibodies (mAb) or test plasma (1:50) were made at 2x concentration and 175 μ l of each of the (depending on the size of the assay) mAb/plasma dilution were added to 175 μ l of target cells. The mixture was incubated for 30 minutes at 4°C . in an Eppendorf tube. Cells were washed once to free unbound antibodies. At this time, 100 μ l of freshly isolated effector cells were added to each well of the 96 well plate

along with 100 μ l of monoclonal antibodies or test plasma bound target cells and incubated at 37°C . and 5% CO_2 for 4 hrs. Samples were tested in duplicates. 100 μ l 2N HCl were added to the target wells for maximum release and 100 μ l of media were added to the target wells for spontaneous release. Specific lysis was calculated as follows: Percent release = $(\text{ER}-\text{SR})/(\text{MR}-\text{SR}) \times 100$ where ER (effectors+target cells release) was experimental release, SR (target cells alone incubated with media) was spontaneous release, and MR (target cells alone incubated with 2N HCl) was maximum release. Percent specific lysis was calculated by subtracting irrelevant target (PC3) release from antigen specific target (LNCaP) release.

TABLE 6

Antibody dependent cytotoxicity activity measured in plasma from human PSMA modified vaccinated animal.

Targets	Percent specific lysis at E:T of 30:1 (based on type of antibodies and targets used in an ADCC assay)			
	Herceptin	Rituxan	Pre-immune plasma (naive)	Post-immune plasma
LNCaP	56.14	8.99	0	49.9
PC3	8.04	0	0	0

Results.

The data from the antibody dependent cytotoxicity assay are presented in Table 6. LNCaP, a human prostate PSMA+ cancer cell line coated with immune plasma derived from the hPSMA immunized animal, was lysed by effector cells while PC3, a human prostate PSMA-cancer cell line coated with the same immune serum, was not lysed by effector cells. Similarly, LNCaP coated with pre-immune plasma was not lysed by effector cells. Herceptin, a monoclonal antibody against HER-2 was used as a positive control since LNCaP cells are known to express HER-2 (Li, Cozzi et al. 2004). Rituxan, a monoclonal against B cell antigen (CD20) was used as a negative control antibody since LNCaP cells do not express CD20. Both monoclonal antibodies are reported to have ADCC activities (Dall'Ozzo, Tartas et al. 2004; Collins, O'Donovan et al. 2011).

Example 2

Construction of PSMA Shuffled Antigens

This example illustrates the construction and certain biological properties of various immunogenic PSMA polypeptides that are variants of the human PSMA modified antigen (SEQ ID NO:9) as described in Example 1.

2A. Design of PSMA Shuffled Antigens

Various immunogenic PSMA polypeptides that are variants of the human PSMA modified antigen (SEQ ID NO:9) as described in Example 1 were designed. These variants were created by introducing mutations selected from orthologs of the human PSMA into the human PSMA modified antigen sequence. These variants are referred to, interchangeably, as "PSMA shuffled antigens" or "shuffled PSMA modified antigens" in the disclosure. The principle and procedure used in creating these variants are provided below.

A computational algorithm was written to select point mutations for the shuffled variant. First, a multiple sequence alignment of PSMA and 12 orthologs (Appendix 2a) was assembled using NCBI's PSI-BLAST. The output from PSI-BLAST included propensities for each residue at each

51

PSMA position among the orthologs. The perl script then used these propensities to select point mutations as follows:

1) Among all positions, the most commonly observed residue is selected that does not match the identity in the native human PSMA.

2) Verify that this mutation position does not overlap with identified Class I or II human PSMA epitopes to ensure that the point mutation is not within a conserved T cell epitope as defined herein above (Table 19).

3) Calculate similarity of mutation to the human residue via the BLOSUM62 matrix to verify that the BLOSUM62 similarity score for the residue substitution is within the range of 0-1 (inclusive).

This iterative procedure is followed until a certain percent sequence identity (below 100) is reached with respect to the human PSMA.

To serve as the input to this algorithm, the PSMA orthologs were assembled to construct a position-specific probability matrix using PSI-BLAST from NCBI. Additionally, the identified epitope regions of PSMA were listed in a file which was also provided to the shuffle algorithm. The non-shuffling regions were also extended to the cytosolic and transmembrane regions of the protein to avoid membrane-bound functionality problems. The orthologous PSMA protein sequences, BLOSUM62 matrix, and PSI-BLAST program were downloaded from the NCBI site.

The shuffling script was then run using these input data and produced a variant of human PSMA with 94% sequence identity with the original human PSMA. Additionally, three mutations to improve HLA-A2 binding were introduced based on their performance in the Epitoptimizer algorithm (Houghton, Engelhorn et al. 2007). These mutations are M664L (epitope: 663-671), 1676V (epitope: 668-676), and N76L (epitope: 75-83). The resultant antigen is referred to as “shuffled PSMA modified antigen 1,” “shuffled PSMA modified 1,” or “PSMA shuffled antigen 1”.

Results based on epitopes with consensus rank <1% and IC50 by neural Network (single best method)<500 showed that predicted epitopes from HLA A2.1, HLA A3, HLA A11, HLA A24, and HLA B7 were highly conserved in this shuffled antigen. Two additional variants of the human PSMA modified antigen described in Example 1 were designed ‘with higher sequence identities and a more restrictive BLOSUM score cutoff of 1 to remove all non-conservative substitutions. These two variants are also referred to as “shuffled PSMA modified antigen 2” and “shuffled PSMA modified antigen 3,” respectively. Percent identities of shuffled PSMA modified antigens 1-3 with respect to the human PSMA modified construct (e.g., amino acids 15-750 of the human PSMA) are approximately 93.6%, 94.9%, and 96.4%, respectively.

The shuffled PSMA modified antigen 1 has the amino acid sequence of SEQ ID NO:3 and has the following mutations relative to the human PSMA modified antigen:
N47S, T53S, K55Q, M58V, L65M, N76L, S98A, Q99E, 55
K122E, N132D, V154I, I157V, F161Y, D191E, M192L,
V201L, V225I, I258V, G282E, I283L, R320K, L362I,
S380A, E408K, L417I, H475Y, K482Q, M509V, S513N,
E542K, M583L, N589D, R598Q, S613N, I614L, S615A,
Q620E, M622L, S647N, E648Q, S656N, I659L, V660L,
L661V, M664L, I676V

The shuffled PSMA modified antigen 2 has the amino acid sequence of SEQ ID NO:5 and has the following mutations relative to the human PSMA modified antigen:

Mutations:
N47S, K55Q, M58V, Q91E, S98A, A111S, K122E, N132D,
V154I, I157V, F161Y, V201L, V225I, I258V, S312A,

52

R320K, K324Q, R363K, S380A, E408K, H475Y, K482Q, Y494F, E495D, K499E, M509L, N540D, E542K, N544S, M583I, I591V, R598Q, R605K, S613N, S647N, E648Q, S656N, V660L

5 The shuffled PSMA modified antigen 3 has the amino acid sequence of SEQ ID NO:7 and has the following mutations relative to the human PSMA modified antigen:

Mutations:

10 T339A, V342L, M344L, T349N, N350T, E351K, S401T, E408K, M470L, Y471H, H475Y, F506L, M509L, A531S, N540D, E542K, N544S, G548S, V555I, E563V, V603A, R605K, K606N, Y607H, D609E, K610N, I611L

15 2B. Immune Responses Measured Post Vaccination in Pasteur Mice

Study Design.

Eight- to 10-week old Pasteur mice were immunized using PMED method with the various plasmid DNAs expressing shuffled PSMA modified antigens in a prime/boost/boost regimen, two weeks apart between each vaccination as described in Example 1F. Antigen specific T and B cell responses were measured 7 days after the last immunization in an interferon-gamma (IFN γ) ELISPOT assay and sandwich ELISA respectively.

TABLE 7

T cell responses induced by various shuffled PSMA modified antigens to peptide pools in PSMA peptide library					
Amino acid	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)				
sequence coverage of target peptide pools from PSMA peptide library	Human PSMA modified antigen	Shuffled PSMA modified antigen1	Shuffled PSMA modified antigen 2	Shuffled PSMA modified antigen 3	
35	13-35	608 (31)	135 (4)	539 (33)	339 (16)
40	109-131	132 (14)	97 (21)	49 (13)	360 (40)
	121-243	275 (7)	146 (17)	104 (17)	542 (119)
	145-167	218 (6)	158 (8)	98 (8)	505 (16)
	157-179	212 (14)	293 (24)	800 (0)*	800 (0)*
	169-191	50 (11)	52 (14)	814 (23)	804 (65)
	181-203	415 (33)	8 (0)	243 (18)	103 (1)
	205-227	125 (7)	1 (1)	17 (7)	7 (4)
	217-239	883 (47)	302 (0)	467 (52)	538 (14)
	229-251	565 (24)	188 (23)	150 (17)	460 (122)
	265-287	418 (8)	2 (0)	154 (25)	168 (23)
	277-299	908 (79)	132 (3)	574 (25)	670 (74)
	289-311	417 (27)	20 (8)	260 (3)	374 (51)
	409-431	377 (7)	61 (10)	38 (3)	48 (11)
	421-443	720 (34)	110 (9)	720 (17)	38 (17)
	433-455	974 (51)	211 (16)	800 (0)*	771 (52)
	481-503	400 (59)	0 (0)	116 (6)	100 (34)
	589-611	60 (14)	245 (30)	679 (35)	364 (11)
	601-623	70 (9)	79 (13)	344 (20)	27 (1)
	613-635	629 (41)	102 (11)	772 (93)	634 (9)
	637-659	226 (17)	292 (0)	539 (16)	420 (198)
	649-671	530 (74)	319 (16)	614 (20)	644 (65)
60	661-683	507 (52)	248 (9)	330 (3)	661 (16)

Results.

ELISpot data presented in Table 7 demonstrates that overall the shuffled PSMA modified antigens are capable of inducing T cell responses in breadth and magnitude very similar to the human PSMA modified antigen. SFC >100 is considered positive. The “**” symbol represents too numerous to accurately count.

TABLE 8

T cell responses induced by shuffled PSMA modified antigens to HLA A2 targets and PSMA protein.				
IFN γ ELISPOT target/antigen	IFN- γ SFC/1 $\times 10^6$ splenocytes(SD)			
	Human PSMA modified antigen	Shuffled PSMA modified antigen 1	Shuffled PSMA modified antigen 2	Shuffled PSMA modified antigen 3
PSMA p168	261 (21)	337 (16)	800+ (0)	800+ (0)
PSMA p275	540 (66)	2 (2)	181 (17)	134 (17)
PSMA p663	441 (46)	152 (10)	219 (6)	600 (48)
HER-2 p106	1 (1)	1 (1)	1 (1)	1 (1)
PSMA ECD protein	839 (70)	165 (31)	569 (44)	319 (6)
BSA protein	1 (1)	1 (1)	1 (1)	2 (0)
LNCaP	229 (45)	40 (3)	137 (23)	45 (6)
MeWo	3 (1)	1 (1)	2 (2)	1 (1)
MeWo-Ad-hPSMA	365 (33)	31 (10)	341 (46)	415 (128)

As shown in Table 8, all the shuffled PSMA antigens are capable of inducing T cells that recognized known HLA A2 restricted PSMA epitopes as well as human HLA A2 tumor cells transduced with adenovirus bearing the PSMA transgene to express PSMA. The tumor cells that did not express PSMA served as negative controls and were not recognized. SFC>50 is considered positive.

TABLE 9

T cell responses induced by shuffled PSMA modified antigens specific to CD4 T cells.				
aa sequence	IFN- γ SFC/1 $\times 10^6$ CD8 depleted splenocytes(SD)			
coverage of target peptide pools from PSMA peptide library	Human PSMA modified antigen	Shuffled PSMA modified antigen 1	Shuffled PSMA modified antigen 2	Shuffled PSMA modified antigen 3
17-31	396 (51)	49 (33)	294 (3)	209 (13)
281-295	528 (6)	51 (1)	592 (9)	461 (24)
285-299	512 (34)	55 (18)	529 (38)	471 (38)
429-443	552 (51)	51 (1)	524 (17)	0
581-595	11 (1)	0	199 (24)	0

ELISpot data shown in Table 9 were obtained with splenocytes that were depleted of CD8; therefore the data represents T cell responses to specific to CD4 T cells. The data show that the CD4 response elicited by shuffled PSMA modified antigen 2 is very similar to that induced by the human PSMA modified antigen. SFC>50 is considered positive.

TABLE 10

Induction of anti-PSMA antibody response as measured by an ELISA assay			
Antigen	ELISA (OD = 0.5)	# of positive	
Human PSMA modified antigen	1159 (802)	7/7	
Shuffled PSMA modified antigen 1	899 (1016)	4/7	
Shuffled PSMA modified antigen 2	4898 (3636)	6/6	
Shuffled PSMA modified antigen 3	1482 (3092)	1/5	

Data in Table 10 demonstrates that all the shuffled PSMA modified antigens are capable of inducing anti-human PSMA antibody responses. Shuffled PSMA modified antigen 2 and the human PSMA modified antigen induced consistent antibody responses in all mice.

TABLE 11

T cell responses in HLA A24 mice induced by the human PSMA modified antigen and shuffled PSMA modified antigen 2.				
Amino acid sequence	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)			
	coverage of target peptide pools from PSMA peptide library	Human PSMA modified antigen	Shuffled PSMA modified antigen 2	
217-231		280 (10)	271 (7)	
233-247		219 (2)	0	
237-251		197 (1)	228 (1)	
249-263		203 (1)	28 (1)	
273-287		57 (2)	323 (3)	
277-291		194 (24)	337 (18)	
293-307		147 (6)	379 (11)	
309-323		17 (1)	441 (14)	
401-415		256 (1)	292 (3)	
429-443		255 (7)	0	
433-447		59 (1)	179 (6)	
481-495		167 (11)	475 (21)	
557-571		194 (14)	297 (1)	
601-615		500 (35)	166 (10)	
605-619		500 (28)	143 (7)	
613-627		218 (4)	0	
697-711		0	141 (8)	
729-743		0	140 (1)	
733-747		0	271 (3)	
737-750		0	401 (8)	

ELISpot data in Table 11 demonstrates that overall the T cell response induced by shuffled PSMA modified antigen 2 in HLA A24 mice is very similar in breadth and magnitude to the human PSMA modified antigen. SFC >100 is considered positive.

2C. Breaking of Immune Tolerance to Human PSMA by Shuffled PSMA Modified Antigens

Study Design.

The human PSMA transgenic mouse model uses mice that were made using the minimal rat probasin promoter driving the expression of PSMA specifically in the prostate gland (Zhang, Thomas et al. 2000) Endocrinology 141(12): 4698-4710. These mice were made in the C57BL/6 background.

RT-PCR and immune histochemistry staining data confirmed the expression of PSMA in the ventral and dorsolateral roots of the prostate gland in these PSMA transgenic mice. The endogenous expression of human PSMA protein in these mice is expected to generate immune tolerance.

Results.

As shown in Table 12, only 20% of the PSMA transgenic mice were able to mount a T cell response to human PSMA using the human PSMA modified antigen. However, 67% of the PSMA transgenic mice were able to mount a PSMA specific T cell response using the shuffled PSMA modified antigen 2. The data suggests that the inclusion of non-self amino acid sequences in the shuffled PSMA modified antigen 2 improved the breaking tolerance to the self human PSMA antigen. SFC>50 is considered positive.

TABLE 12

Antigen	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)									# of positive
	7 (3)	126 (20)	1 (2)	3 (1)	10 (5)	22 (5)	30 (3)	12 (0)	7 (2)	
Human PSMA modified antigen										
Shuffled PSMA modified antigen 2	474 (52)	104 (18)	7 (3)	111 (8)	12 (2)	21 (2)	139 (12)	246 (9)	226 (11)	NA 6/9

Example 3

Design of Various Immunogenic PSA Polypeptides

Example 3 illustrates the construction and certain biological properties of immunogenic PSA polypeptides in cytosolic, secreted, and membrane-bound forms.

3A. Construction of Various PSA Antigen Forms

Similar to what was described in Example 1 for the three different immunogenic PSMA polypeptide forms (e.g., the cytosolic, membrane-bound, and secreted forms), immunogenic PSA polypeptides in the three forms were also designed based on the human PSA sequence. An immunogenic PSA polypeptide in cytosolic form, which consists of amino acids 25-261 of the native human PSA, is constructed by deleting the secretory signal and the pro domain (amino acids 1-24). The amino acid sequence of this cytosolic immunogenic PSA polypeptide is provided in SEQ ID NO: 17. The secreted form of the PSA polypeptide is the native full length human PSA (amino acids 1-261). An immunogenic PSA polypeptide in membrane-bound form is constructed by linking the immunogenic PSA polypeptide cytosolic form (amino acids 25-261 of the native human PSA) to the human PSMA transmembrane domain (amino acids 15-54 of the human PSMA).

3B. Immune Responses in Pasteur and HLA A24 Mice Study Design.

Eight to 10 week old HLA A2 Pasteur mice or HLA A24 mice were immunized with DNA expressing the various PSA antigens using PMED provided in Example 3A in a prime/boost/boost regimen with two week intervals between each vaccination as described in Example 1. The antigen specific T and B cell responses were measured 7 days after the last immunization in an interferon-gamma (IFN γ) ELISPOT assay and sandwich ELISA.

TABLE 13

Induction of T cell responses in Pasteur mice and HLA A24 mice vaccinated with PSA polypeptides			
Amino acid sequence coverage of target	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)		
	peptide pools from PSA peptide library	PSA cytosolic	PSA membrane-bound
T cell response detected in HLA A2 Pasteur mice			
25-47	12 (0)	134 (6)	27 (1)
49-71	150 (0)	23 (1)	151 (10)
61-83	904 (17)	27 (7)	452 (14)
73-95	128 (25)	8 (6)	78 (14)

TABLE 13-continued

Induction of T cell responses in Pasteur mice and HLA A24 mice vaccinated with PSA polypeptides			
Amino acid sequence coverage of target	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)		
	peptide pools from PSA peptide library	PSA cytosolic	PSA membrane-bound
85-107	17 (7)	205 (18)	11 (1)
97-117	26 (3)	378 (25)	13 (1)
217-239	16 (8)	234 (8)	6 (6)
229-251	96 (34)	844 (6)	35 (12)
T cell response detected in HLA A24 mice			
145-167	357 (2)	Not determined	Not determined

Results.

Table 13 shows ELISpot data derived from splenocytes isolated from HLA A2 Pasteur mice or HLA A24 mice cultured with peptides derived from the PSA peptide library. T cell responses can be detected in both HLA A2 and HLA A24 mice. SFC>100 is considered positive.

TABLE 14

The induction of T cell responses by PSA antigens in Pasteur mice to PSA+ HLA A2.1+ SKmel5 human cancer cells			
HLA A2.1+ human cancer cells or protein	IFN- γ SFC/1 $\times 10^6$ splenocytes (SD)		
	PSA cytosolic	PSA membrane-bound	PSA secreted
SKmel5-Ad-eGFP	7.7 (9.6)	1.2 (1.4)	2.9 (2.7)
SKmel5-Ad-PSA	112.0 (169.3)	546.1 (379.6)	18.7 (18.5)
PSA protein	108.8 (161.0)	536.9 (380.9)	20.6 (21)

ELISpot data shown in table 14 indicates that immunogenic PSA polypeptides in both cytosolic and membrane-bound forms are capable of inducing T cells that recognize human tumor cells transduced with adenovirus to express the cytosolic PSA antigen (SKmel5-Ad-PSA) but not cells transduced with adenovirus to express eGFP (SKmel5-Ad-eGFP). These two antigens also elicited response to PSA protein. The PSA secreted antigen failed to induce T cells to both SKmel5-Ad-PSA or PSA protein. SFC>50 is considered positive.

TABLE 15

The induction of anti-PSA antibody response as measured by a sandwich ELISA assay

Antigen Forms	ELISA (OD = 1.0) Average (SD)	# of positive
PSA cytosolic	99 (0)	0/6
PSA membrane-bound	4838 (835)	6/6
PSA secreted	1151 2410)	2/6

Data in Table 15 demonstrates that immunogenic PSA polypeptides in both secreted and membrane-bound forms are capable of inducing anti-PSA antibody responses.

TABLE 16

Human PSMA Peptide Library peptide pools and corresponding amino acid sequences	
PSMA peptide pool (aa no.)	aa sequences of individual peptides

1-23	MWNL LHETD SAVATA LHETD SAVATARRPR DSAVATARRPRWLCA	5	169-191	MPEGDLVYVNYARTE DLVYVNYARTEDFFK VNYARTEDFFKLERD
13-35	ATARRPRWLCA GAGALV RPRWLCA GAGALVLAGG LCAGALVLAGGFLL	10	181-203	RTEDFFKLERDMKIN FFKLERDMKINC SGK ERDMKINC SGKIVIA
25-47	ALVLAGGFLLGFLF AGGFFL LGFL FGWF FLLGFL FGWF IKSSN	15	193-215	KINC SGKIVIARYGK SGKIVIARYGKVFRG VIARYGKVFRGNKVK
37-59	PLFGWFI KSSNEATN WFI KSSNEATNITPK SSNEATNITPKHNMK	20	205-227	YGVFRGNKVKNAQL FRGNKVKNAQLAGAK KVKNAQLAGAKGVIL
49-71	ATNITPKHNMK AFLD TPKHNMK AFLDELKA NMKAFLDELKAENIK	25	217-239	AQLAGAKGVILYSDP GAKGVILYSDPDADYF VILYSDPDADYFAPGV
61-83	FLDELKAENIKKFLY LKAENIKKFLYNF TQ NIKKFLYNF TQIPHL	30	229-251	SDPDADYFAPGVKSYP DYFAPGVKSYPDGWN PGVKSYPDGWNLPGG
73-95	FLYNFTQIPHLAGTE FTQIPHLAGTEQN FQ PHLAGTEQN PQLAKQ	35	241-263	SYPDGWNLPGGGVQR GWNLPGGGVQRGNIL PGGGVQRGNILNLNG
85-107	GTEQNF QOLAKQI QSQ NQOLAKQI QSQWKEF AKQI QSQWKEF GLDS	40	253-275	VQRGNI LNLNGAGDP NLNLNGAGDPLTPG LNGAGDPLTPGY PAN
97-117	QSQWKEF GLDS VELA KEF GLDS VELAH YDV LDS VELAH YDV LSY	45	265-287	GDPLTPGY PAN EYAY TPGY PAN EYAY RGI PANEYAY RRGIAE AV
109-131	ELAH YDV LLS YPNKT YDV LLS YPNK THPNY LSPN KTHPN YISII	50	277-299	YAYRRGIAE AVGLPS RGIAE AVGLPS IPVH EA VGLPS IPVHP IGY
121-143	NKTHPN YISII INEDG PNY YISII NEDGNE IF SI INEDGNE IFNTSL	55	289-311	LPSIPVHP IGY DAQ PVHP IGY DAQ KLL IGYYDAQ KLL EKMG
133-155	EDGNE IFNTSL FEP EIFNTSL FEP PPP PGY TSL FEP PPP PGY ENVS	60	301-323	DAQ KLL EKMGGS APP LLEKMGGS APP DSSW MGGS APP DSS WRG SL
145-167	EPPPPG YEN VSDIV P PGY EN VSDIV PPF SA NVSDIV PPF SA FSPQ	65	313-335	APPDSSWRG SL KV PY SSWRG SL KV PY NV GP GSL KV PY NV GP GFTG
157-179	IVPPF SAFS POGMPE FSAFS PQGM PEGD LV SPQGM PEGD LV YV NY	70	325-347	VPY NVGP GFTG NF ST VGP GFTG NF STQ KV K FTG NF STQ KV KM HI H
			337-359	FSTQ KV KM HI H ST NE KV KM HI H ST NE VTR I HI H ST NE VTR I YN VI
			349-371	TNEVTR I YN VIG TL R TRI YN VIG TL RGA VE NVIG TL RGA VE PDRY
			361-383	TL RGA VE PDRY VIL G A VEPDRY VIL GG HRD DRY VIL GG HRD SW V
			373-395	I LGG HRD SW VFGG ID HRD SW VFGG ID P QSG WVFGG ID P QSG AAV V

TABLE 16 -continued

59

TABLE 16-continued

Human PSMA Peptide Library peptide pools and corresponding amino acid sequences	
PSMA peptide pool (aa no.)	aa sequences of individual peptides
385-407	GIDPQSGAAVVHEIV QSGAAVVHEIVRSFG AVVHEIVRSFGTLKK
397-419	EIVRSFGTLKKEGWR SFGTLLKEGWRPRRT LKKEGWRPRRTILFA
409-431	GWRPRRTILFASWDA RRTILEFASWDAEEFG LFASWDAEEFGLLGS
421-443	WDAEEFGLLGSTEWA EFGLLGSTEWAENS LGSTEWAEENSRLLQ
433-455	EWAEENSRLLQERGV ENSRLLQERGVAYIN LLQERGVAYINADSS
445-467	RGVAYINADSSIEGN YINADSSIEGNYTLR DSSIEGNYTLRVDC
457-479	EGNYTLRVDCTPLMY TLRVDCTPLMYSLVH DCTPLMYSLVHNLT
469-491	LMSVLVHNLTKEKLS LVHNLTKEKSPDEG LTKEKSPDEGFEGK
481-503	LKSPDEGFEGKSLYE DEGFEGKSLYESWTK EGKSLYESWTKKSPS
493-515	LYESWTKKSPPEFS WTKKSPPEFSGMPR SPSPEFSGMPRIKSL
505-527	EFGMPRIKLGSGN MPRIKLGSGNDFEV SKLGSGNDFEVFKQR
517-539	SGNDFEVFFQRLGIA FEVFFFQRLGIAASGRA FQRLGIAASGRARYTK
529-551	GIASGRARYTKNWET GRARYTKNWETNKFS YTWNWETNKFGYPL
541-563	WETNKFGYPLYHSV KFSGYPLYHSVYETY YPLYHSVYETYELVE
553-575	HHSVYETYELVEKFYD ETTYELVEKFYDPMFK LVEKFYDPMFKYHLT
565-587	FYDPMPFKYHLTVAQV MFKYHLTVAQVRGGM HLTVAVQRGGMVFEL
577-599	AQVRGGMVFELANSI GGMVfelansivlpf FELANSIVLPFDRC
589-611	NSIVLPFDRCRDYAVV LPFDRCRDYAVVLRKY CRDYAVVLRKYADKI

60

TABLE 16-continued

Human PSMA Peptide Library peptide pools and corresponding amino acid sequences	
PSMA peptide pool (aa no.)	aa sequences of individual peptides
601-623	AVVLRKYADKIYSIS RKYADKIYSISMKHP DKIYSISMKHPQEMK
613-635	SISMKHPQEMKTYSV KHPQEMKTYSVSFDS EMKTYSVSFDSLFS
625-647	YSVSFDSLFSAVKNF FDSLFSAVKNFTEIA FSAVKNFTEIASKFS
637-659	KNFTEIASKFSERLQ EIASKFSERLQDFDK KFSERLQDFDKSNPI
649-671	RLQDFDKSNPIVLRM FDKSNPIVLRMMNDQ NPIVLRMMNDQLMFL
661-683	LRMMNDQLMFLERA NDQLMFLERAIDPL MFLERAIDPLGLPD
673-695	RAFIDPLGLPDRPFY DPLGLPDRPFYRHVI LPDRPFYRHVIYAPS
685-707	PFYRHVIYAPSSHNK HVIYAPSSHNKYAGE APSSHNKYAGESFPG
697-719	HNKYAGESFPGIYDA AGESFPGIYDALFDI FPGIYDALFDIESKV
709-731	YDALFDIESKVDPSK FDIESKVDPSKAWGE SKVDPSKAWGEVKRQ
721-743	PSKAWGEVKRQIYVA WGEVKRQIYVAAFTV KRQIYVAAFTVQAAA
733-750	YVAAFTVQAAAETLS FTVQAAAETLSEVA
Human PSA Peptide Library	
PSA peptide pool (aa no.)	aa sequences of individual peptides
1-23	MWVPVVFLTLSVTWI VVPLTLSVTWIGAAP TLSVTWIGAAPLILS
13-35	TWIGAAPLILSRIVG AAPLILSRIVGGWEC ILSRIVGGWECEKHS
25-47	IVGGWECEKHSQPWQ WECEKHSQPWQVLVA KHSQPWQVLVSRGR
37-59	PWQVLVAVSRGRAVCG LVASRGRAVCGGLV RGRAVCGGLVHPQW

TABLE 17

US 9,468,672 B2

61

TABLE 17-continued

Human PSA Peptide Library	
PSA peptide pool (aa no.)	aa sequences of individual peptides
49-71	VCGGVLVHPQWLTA VLVHPQWLTAAHCI PQWLTAAHCIRNKS
61-83	LTAAHCIRNKSILL HCIRNKSILLGRHS NKSILLGRHSLFHP
73-95	ILLGRHSLFHPEDTG RHSLFHPEDTGQVFQ FHPEDTGQVFQVSHS
85-107	DTGQVFPQVSHSFPHP VFQVSHSFPHPLYDM SHSFPHPLYDMSLLK
97-117	PHPLYDMSLLKNRFL YDMSLLKNRFLRPGD LLKNRFLRPGDDSSH
109-131	RFLRPGDDSSHDLML PGDDSSHDLMLRLS SSHDLMLRLRSEPAE
121-243	LMLLRLEPAELTDA RLSEPAELTDAVKVM PAELTDAVKVMDLPT
133-155	TDAVKVMDLPTQEPA KVMDLPTQEPAALGTT LPTQEPALEGTCYAS
145-167	EPALGTTCYASGWGS GTTCYASGWGSIEPE YASGWGSIEPEEFLT
157-179	WGSIEPEEFLTPKKL EPEEFLTPKKLQCVD FLTPKKLQCVDLHVI
169-191	KKLQCVDLHVISNDV CVDLHVISNDVCAQV HVISNDVCAQVHPQK
181-203	NDVCAQVHPQKVTKF AQVHPQKVTKFMLCA PKVTKFMLCAGRWT
193-215	TKFMLCAGRWTGGKS LCAGRWTGGKSTCSG RWTGGKSTCSGDSGG
205-227	GKSTCGDSGGPLVC CGDSGGPLVCNGVL SGGPLVCNGVLQGIT
217-239	LVCNGVLQGITSWGS GVLQGITSWGEPCA GITSWGSEPCALPER
229-251	WGSEPCALPERPSLY PCALPERPSLYTKVV PERPSLYTKVHYRK
241-263	SLYTAKVHYRKWIKD KVVHYRKWIKDITIVA YRKWIKDITIVANP

62

TABLE 18

PSMA Orthologs			
	PSMA species	NCBI ID	% ID with human
5	human	2897946	100
	chimpanzee	114639743	99
	macaque	109108238	97
	dog	73987958	93
	horse	149719573	92
	pig	47523822	90
	cow	156120365	89
	rat	149069047	84
	mouse	20138153	84
	opossum	126327828	80
10	chicken	118085215	78
	platypus	149635150	76
	zebra fish	41053648	69

TABLE 19

Conserved T Cell Epitopes in the Human PSMA as Set Forth in SEQ ID NO: 1.				
	Amino acid Start	Amino acid End	Sequence	
20	168	176	GMPEGDLVY	
	347	356	HSTNGVTRIY	
25	557	566	ETYELVEKFY	
	207	215	KVFRGNKVK	
30	431	440	STEWAEEENSR	
	4	12	LLHETDSAV	
35	27	35	VLAGGFPLL	
	168	177	GMPEGDLVYV	
40	441	450	LLQERGVAYI	
	469	477	LMSVLVHNL	
45	711	719	ALFDIESKV	
	663	671	MNDQVMPL	
50	178	186	NYARTEDFF	
	227	235	LYSDPADYF	
55	624	632	TYSVSFDSL	
	334	348	TGNFSTQKVKMHIHS	
60	459	473	NYTLRVDCPLMYSL	
	687	701	YRHVIYAPSSHNKYA	
65	730	744	RQIYVAAFTVQAAA	

Example 4

Construction of Multi-Antigen Vaccine Constructs

In this Example, several strategies for expressing multiple antigens from single component DNA vaccine construct are described. These multi-antigen DNA vaccine constructs share the same general plasmid backbone as pPJ7563. Although the multi-antigen expression strategies are described here in the context of a DNA vaccine, the principles will apply similarly in the context of viral vector

genetic vaccines (such as adenovirus vectors). Unless otherwise specified, the genes included in the multi-antigen constructs encode the human PSMA modified antigen (noted as PSMA), full length human PSCA (noted as PSCA), and the human PSA cytosolic antigen (noted as PSA), as described in the examples herein above.

Example 4A

Dual Antigen Constructs

4A1. Construction of Dual Antigen Constructs Utilizing Multiple Promoters

General Strategy.

One strategy for creating multivalent nucleic acid vaccine constructs is to incorporate multiple independent promoters into a single plasmid (Huang, Y., Z. Chen, et al. (2008). "Design, construction, and characterization of a dual-promoter multigenic DNA vaccine directed against an HIV-1 subtype C/B' recombinant." *J Acquir Immune Defic Syndr* 47(4): 403-411; Xu, K., Z. Y. Ling, et al. (2011). "Broad humoral and cellular immunity elicited by a bivalent DNA vaccine encoding HA and NP genes from an H5N1 virus." *Viral Immunol* 24(1): 45-56). The plasmid can be engineered to carry multiple expression cassettes, each consisting of a) a eukaryotic promoter for initiating RNA polymerase dependent transcription, with or without an enhancer element, b) a gene encoding a target antigen, and c) a transcription terminator sequence. Upon delivery of the plasmid to the transfected cell nucleus, transcription will be initiated from each promoter, resulting in the production of separate mRNAs, each encoding one of the target antigens. The mRNAs will be independently translated, thereby producing the desired antigens.

Plasmid 460 (PSMA/PSCA Dual Promoter).

Plasmid 460 was constructed using the techniques of site-directed mutagenesis, PCR, and restriction fragment insertion. First, a Kpn I restriction site was introduced upstream of the CMV promoter in plasmid 5259 using site-directed mutagenesis with MD5 and MD6 primers according to manufacturer's protocol (Quickchange kit, Agilent Technologies, Santa Clara, Calif.). Second, an expression cassette consisting of a minimal CMV promoter, human PSMA, and rabbit B globulin transcription terminator was amplified by PCR from plasmid 5166 using primers that carried Kpn I restriction sites (MD7 and MD8). The PCR amplicon was digested with Kpn I and inserted into the newly introduced Kpn I site of calf intestinal alkaline phosphatase (CIP)-treated plasmid 5259.

4A2. Construction of Dual Antigen Constructs Utilizing 2A Peptides

General Strategy.

Multiple protein antigens can also be expressed from a single vector through the use of viral 2A-like peptides (Szymczak, A. L. and D. A. Vignali (2005). "Development of 2A peptide-based strategies in the design of multicistronic vectors." *Expert Opin Biol Ther* 5(5): 627-638; de Felipe, P., G. A. Luke, et al. (2006). "E unum pluribus: multiple proteins from a self-processing polyprotein." *Trends Biotechnol* 24(2): 68-75; Luke, G. A., P. de Felipe, et al. (2008). "Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes." *J Gen Virol* 89 (Pt 4): 1036-1042; Ibrahim, A., G. Vande Velde, et al. (2009). "Highly efficient multicistronic lentiviral vectors with peptide 2A sequences." *Hum Gene Ther* 20(8): 845-860; Kim, J. H., S. R. Lee, et al. (2011). "High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell

lines, zebrafish and mice." *PLoS One* 6(4): e18556). These peptides, also called cleavage cassettes or CHYSELs (cis-acting hydrolase elements), are approximately 20 amino acids long with a highly conserved carboxy terminal D-V/I-EXNP GP motif (FIG. 2). The cassettes are rare in nature, most commonly found in viruses such as Foot-and-mouth disease virus (FMDV), Equine rhinitis A virus (ERAV), Encephalomyocarditis virus (EMCV), Porcine teschovirus (PTV), and Thosea asigna virus (TAV) (Luke, G. A., P. de Felipe, et al. (2008). "Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes." *J Gen Virol* 89 (Pt 4): 1036-1042). With a 2A-based multi-antigen expression strategy, genes encoding multiple target antigens can be linked together in a single open reading frame, separated by 2A cassettes. The entire open reading frame can be cloned into a vector with a single promoter and terminator. Upon delivery of the genetic vaccine to a cell, mRNA encoding the multiple antigens will be transcribed and translated as a single polyprotein. During translation of the 2A cassettes, ribosomes skip the bond between the C-terminal glycine and proline. The ribosomal skipping acts like a cotranslational autocatalytic "cleavage" that releases upstream from downstream proteins. The incorporation of a 2A cassette between two protein antigens results in the addition of ~20 amino acids onto the C-terminus of the upstream polypeptide and 1 amino acid (proline) to the N-terminus of downstream protein. In an adaptation of this methodology, protease cleavage sites can be incorporated at the N terminus of the 2A cassette such that ubiquitous proteases will cleave the cassette from the upstream protein (Fang, J., S. Yi, et al. (2007). "An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies in vivo." *Mol Ther* 15(6): 1153-1159).

Plasmid 451 (PSMA-T2A-PSCA).

Plasmid 451 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding human PSMA amino acids 15-750 was amplified by PCR using plasmid 5166 as a template with primers 119 and 117. The gene encoding full-length human PSCA was amplified by PCR using plasmid 5259 as a template with primers 118 and 120. PCR resulted in the addition of overlapping TAV 2A (T2A) sequences at the 3' end of PSMA and 5' end of PSCA. The amplicons were mixed together and amplified by PCR with primers 119 and 120. The PSMA-T2A-PSCA amplicon was digested with Nhe I and Bgl II and inserted into similarly digested plasmid 5166. A glycine-serine linker was included between PSMA and the T2A cassette to promote high cleavage efficiency.

Plasmid 454 (PSCA-F2A-PSMA).

Plasmid 454 was created using the techniques of PCR and restriction fragment exchange. First, the gene encoding full-length human PSCA was amplified by PCR using plasmid 5259 as a template with primers 42 and 132. The amplicon was digested with BamH I and inserted into similarly digested, CIP-treated plasmid 5300. A glycine-serine linker was included between PSCA and the FMDV 2A (F2A) cassette to promote high cleavage efficiency.

Plasmid 5300 (PSA-F2A-PSMA)

Plasmid 5300 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding PSA amino acids 25-261 was amplified by PCR from plasmid 5297 with primers MD1 and MD2. The gene encoding human PSMA amino acids 15-750 was amplified by PCR from plasmid 5166 with primers MD3 and MD4. PCR resulted in the addition of overlapping F2A sequences at the 3' end of PSA and 5' end of PSMA. The amplicons were mixed together and extended by PCR. The

65

PSA-F2A-PSMA amplicon was digested with Nhe I and Bgl II and inserted into similarly digested plasmid pPJ7563.

4A3. Dual Antigen Constructs Utilizing Internal Ribosomal Entry Sites

General Strategy:

A third strategy for expressing multiple protein antigens from a single plasmid or vector involves the use of an internal ribosomal entry site, or IRES. Internal ribosomal entry sites are RNA elements (FIG. 3) found in the 5' untranslated regions of certain RNA molecules (Bonnal, S., C. Boutonnet, et al. (2003). "IRESdb: the Internal Ribosome Entry Site database." *Nucleic Acids Res* 31(1): 427-428). They attract eukaryotic ribosomes to the RNA to facilitate translation of downstream open reading frames. Unlike normal cellular 7-methylguanosine cap-dependent translation, IRES-mediated translation can initiate at AUG codons far within an RNA molecule. The highly efficient process can be exploited for use in multi-cistronic expression vectors (Bochkov, Y. A. and A. C. Palmenberg (2006). "Translational efficiency of EMCV IRES in bicistronic vectors is dependent upon IRES sequence and gene location." *Bio-techniques* 41(3): 283-284, 286, 288). Typically, two transgenes are inserted into a vector between a promoter and transcription terminator as two separate open reading frames separated by an IRES. Upon delivery of the genetic vaccine to the cell, a single long transcript encoding both transgenes will be transcribed. The first ORF will be translated in the traditional cap-dependent manner, terminating at a stop codon upstream of the IRES. The second ORF will be translated in a cap-independent manner using the IRES. In this way, two independent proteins can be produced from a single mRNA transcribed from a vector with a single expression cassette.

Plasmid 449 (PSMA-mIRES-PSCA).

Plasmid 449 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding full length human PSCA was amplified by PCR from plasmid 5259 with primers 124 and 123. The minimal EMCV IRES was amplified by PCR from pShuttle-IRES with primers 101 and 125. The overlapping amplicons were mixed together and amplified by PCR with primers 101 and 123. The IRES-PSCA amplicon was digested with Bgl II and BamH I and inserted into Bgl II-digested, CIP-treated plasmid 5166. In order to fix a spontaneous mutation within the IRES, the IRES containing Avr II to Kpn I sequence was replaced with an equivalent fragment from pShuttle-IRES.

Plasmid 603 (PSCA-pIRES-PSMA).

Plasmid 603 was constructed using the techniques of PCR and seamless cloning. The gene encoding full length human PSCA attached at its 3' end to a preferred EMCV IRES was amplified from plasmid 455 by PCR with primers SD546 and SD547. The gene encoding human PSMA amino acids 15-750 was amplified by PCR from plasmid 5166 using primers SD548 and SD550. The two overlapping PCR amplicons were inserted into Nhe I and Bgl II-digested pPJ7563 by seamless cloning according to manufacturer's instructions (Invitrogen, Carlsbad, Calif.).

Plasmid 455 (PSCA-mIRES-PSA).

Plasmid 455 was constructed using the techniques of overlapping PCR and restriction fragment exchange. First, the gene encoding human PSA amino acids 25-261 was amplified by PCR from plasmid 5297 with primers 115 and 114. The minimal EMCV IRES was amplified by PCR from pShuttle-IRES with primers 101 and 116. The overlapping amplicons were mixed together and amplified by PCR with primers 101 and 114. The IRES-PSA amplicon was digested with Bgl II and BamH I and inserted into Bgl II-digested,

66

CIP-treated plasmid 5259. In order to fix a spontaneous mutation within this clone, the Bgl II to BstE II sequence was replaced with an equivalent fragment from a fresh overlapping PCR reaction.

5

Example 4B

Triple Antigen DNA Constructs

General Strategy.

The abilities of the dual antigen expression vectors to direct the expression of PSMA, PSCA, and/or PSA were characterized in transfected HEK293 cells (FIGS. 4, 5A, 5B, and 6). A number of dual antigen expression cassettes, including PSA-F2A-PSMA, PSMA-mIRES-PSCA, PSMA-T2A-PSCA, PSA-T2A-PSCA, PSCA-F2A-PSMA, PSCA-pIRES-PSMA, and PSMA-mIRES-PSA, were selected for incorporation in various combinations into triple antigen expression vectors. In all cases, the vectors were based on the parental pPJ7563 plasmid backbone. Four vectors (plasmids 456, 457, 458, and 459) utilized a single full CMV promoter with a rabbit B globulin transcription terminator to drive expression of all three antigens. Two other vectors (plasmids 846 and 850) incorporated a dual promoter strategy in combination with either an IRES or 2A to drive expression of the three antigens. Vectors with multiple 2A cassettes were engineered to carry different cassettes to minimize the likelihood of recombination between the first and second cassette during plasmid/vector amplification. Antigen expression was demonstrated by flow cytometry (FIGS. 7A and 7B) and western blotting (FIGS. 8A and 8B).

Plasmid 456 (PSA-F2A-PSMA-mIRES-PSCA).

Plasmid 456 was constructed by restriction fragment exchange. Plasmid 5300 was digested with Nhe I and Hpa I and the ~1.8 kb insert was ligated into similarly digested plasmid 449.

Plasmid 457 (PSA-F2A-PSMA-T2A-PSCA).

Plasmid 457 was constructed by restriction fragment exchange. Plasmid 5300 was digested with Nhe I and Hpa I and the ~1.8 kb insert was ligated into similarly digested plasmid 451.

Plasmid 458 (PSA-T2A-PSCA-F2A-PSMA).

Plasmid 458 was constructed using the techniques of PCR and restriction fragment exchange. The gene encoding human PSA amino acids 25-261 was amplified by PCR from plasmid 5297 with primers 119 and 139, resulting in the addition of a T2A sequence and Nhe I restriction site at the 3' end. The amplicon was digested with Nhe I and inserted into similarly digested plasmid 454.

Plasmid 459 (PSCA-F2A-PSMA-mIRES-PSA).

Plasmid 459 was constructed by restriction fragment exchange. Plasmid 454 was digested with Nhe I and Bgl II and the PSCA-F2A-PSMA containing insert was ligated into similarly digested plasmid 455.

Plasmid 846 (CBA-PSA, CMV-PSCA-pIRES-PSMA).

Plasmid 846 was constructed using the techniques of PCR and seamless cloning. First, an expression cassette was synthesized that consisted of 1) the promoter and 5' untranslated region from the chicken beta actin (CBA) gene, 2) a hybrid chicken beta actin/rabbit beta globin intron, 3) the gene encoding human PSA amino acids 25-261, and 4) the bovine growth hormone terminator. This PSA expression cassette was amplified by PCR from plasmid 796 with primers 3SalICBA and 5SalIBGH. The amplicon was cloned into the SalII site of plasmid 603 using a GeneArt Seamless Cloning and Assembly Kit (Invitrogen, Carlsbad, Calif.). Upon delivery of this plasmid into a cell, PSA expression

50

55

60

65

will be driven off the CBA promoter while PSCA and PSMA expression will be driven off the CMV promoter.

Plasmid 850 (CBA-PSA, CMV-PSCA-F2A-PSMA).

Plasmid 850 was constructed using the techniques of PCR and seamless cloning. First, the CBA promoter-driven PSA expression cassette was amplified by PCR from plasmid 796 with primers 3SalICBA and 5SalIBGH. The amplicon was cloned into the SalI site of plasmid 454 using GeneArt Seamless Cloning. Upon delivery of this plasmid into a cell, PSA expression will be driven off the CBA promoter while PSCA and PSMA expression will be driven off the CMV promoter.

TABLE 20

List of Plasmids Expressing Multiple-Antigens					
1 st Antigen	Expression strategy	2 nd Antigen	Expression strategy	3 rd Antigen	Plasmid ID #
PSMA				5166	5166
PSCA				5259	5259
PSA				5297	5297

TABLE 20-continued

List of Plasmids Expressing Multiple-Antigens						
5	1 st Antigen	Expression strategy	2 nd Antigen	Expression strategy	3 rd Antigen	Plasmid ID #
	PSMA	2 promoters	PSCA			460
	PSMA	T2A	PSCA			451
	PSCA	F2A	PSMA			454
	PSA	F2A	PSMA			5300
	PSMA	IRES	PSCA			449
	PSCA	IRES	PSMA			603
	PSCA	IRES	PSA			455
	PSA	F2A	PSMA	mIRES	PSCA	456
	PSA	F2A	PSMA	T2A	PSCA	457
	PSA	T2A	PSCA	F2A	PSMA	458
	PSCA	F2A	PSMA	mIRES	PSA	459
	PSA	2 promoters	PSCA	pIRES	PSMA	796
	PSA	2 promoters	PSCA	pIRES	PSMA	846
	PSA	2 promoters	PSCA	F2A	PSMA	850

TABLE 21

List of Primers Used in the Construction of the Multi-antigen Plasmids		
Primer	Sequence (5' to 3')	Strand
42	CGTGACGCAAATGGGCGGTAGG	Sense
101	TCAGAGATCTGACCCCTAACGTTACTGGC	Sense
114	TATAGGATCCTCAGGGTGGCACGATG	Antisense
115	GAAAAACACGATGATAATATGGCAGCATGTGGAGGCTGGAGTG	Sense
116	CCACAATGCTGGCCATATTATCATCGTGTGTTCAAAGGAAACCACGT	Antisense
117	CC	Antisense
117	CATCTCACAGGTCAATAATGAACCCCTACCTCGGATCCGGTACTTC	Antisense
117	ACTCAAAGTC	
118	GTTCAATTGACCTGTGGAGATGTCGAAGAAAACCAGGACCCGCAA	Sense
118	GCAAGGCTGTGCTGCTTGCCTG	
119	TTGCCCTCACATCTCGTCAATCTCCGGAGGAC	Sense
120	GATCTTTGTACAATATGATCTTGTGGCAATGTCCC	Antisense
123	TATAGGATCCCTATAGCTGGCCGGGTCC	Antisense
124	CACGATGATAATGCCAGCAAGGCTGTGCTGCTG	Sense
125	CACAGCCTGCTGGCCATATTATCATCGTGTGTTCAAAGGAAACCAC	Antisense
125	GTCC	
132	TATAGGATCCTAGCTGGCCGGGTCCCCAGAG	Antisense
139	ATATGCTAGGGTCTGGGTTCTTCGACATCTCCACAGGTCAATAA	Antisense
139	TGAACCCCTACCTCGGATCCGGGG	
139	TTGGCCACGATGGTGTCC	
SD546	CTGTGACGAAATGGCTAGCAAGG	Sense
SD547	ATTATCATCGTGTGTTCAAAGGAAAACC	Antisense
SD548	AAACACGATGATAATATGCCACACCATGGCGCGCCGCCGC	Sense
SD550	TTTTGTTAGGGCCCAGATCTTAGGC	Antisense
MD1	GACGAACATGGCTAGCATTGTGGGAGGCTG	Sense
MD2	CCACATCGCCCTGCCAGTTTCAGCAGATCAAAGTTCAAGGTCTGGGATC	Antisense
MD2	CGGGGTTGCCACGATGGTGTCC	

TABLE 21-continued

List of Primers Used in the Construction of the Multi-antigen Plasmids		
Primer	Sequence (5' to 3')	Strand
MD3	GATCTGCTGAAACTGGCAGGCATGTGGAAAGCAACCCAGGCCAATG GCAAGCGCGCCGCCGCGCTG	Sense
MD4	GTTAGGGCCCAGATCTTCTAGGCTACTTCAGTCAGTC	Antisense
MD5	CTTGTATTACTGTTATGTAAGCAGACAGGGTACCAATATTGGCTATTG GCCATTGCAATAC	Sense
MD6	GTATGCAATGGCCAATAGCCAATTGGTACCCCTGCTGCTTACATAAA CAGTAATACAAG	Antisense
MD7	CATGCATGGGTACCAATCTCCGAGTGAGAGACACAAAAATTCC	Sense
MD8	GATCGATCGGTACCCCTGCAGGTGAGCACCACAAACACGGG	Antisense
5SalIBGH	GTTCATGTAAGCAGACAGGTCGACCCATAGAGCCCCACCGCATCCCCAGC	Antisense
3SalICBA	TGGCCAATAGCCAATTGTCGACTGGTCGAGGTGAGCCCCACGTTC TG	Sense

Example 4C

Triple Antigen Adenovirus Constructs

General Strategy.

As with DNA plasmids, viral vaccine vectors can be engineered to deliver multiple prostate cancer antigens. The three multi-antigen expression strategies described above for DNA vaccines—dual promoters, 2A peptides, and internal ribosome entry sites—were incorporated in various combinations to create triple antigen adenovirus vectors. Briefly, the multi-antigen expression cassettes were cloned into a pShuttle-CMV plasmid modified to carry two copies of the tetracycline operator sequence (TetO2). Recombinant adenovirus serotype 5 vectors were created using the AdEasy Vector System according to manufacturer's protocols (Agilent Technologies, Inc., Santa Clara, Calif.). Viruses were amplified in HEK293 cells and purified by double cesium chloride banding according to standard protocols. Prior to in vivo studies, viral stocks were thoroughly characterized for viral particle concentration, infectivity titer, sterility, endotoxin, genomic and transgene integrity, transgene identity and expression.

Adenovirus-733 (PSA-F2A-PSMA-T2A-PSCA).

Ad-733 is the viral equivalent of plasmid 457. Expression of the three antigens is driven off a single CMV promoter with a tetracycline operator for repressing transgene expression during large scale production in Tet repressor expressing HEK293 lines. Multi-antigen expression strategies include two different 2A sequences.

Adenovirus-734 (PSA-T2A-PSCA-F2A-PSMA).

Ad-734 is the viral equivalent of plasmid 458. Expression of the three antigens is driven off a single CMV promoter with a tetracycline operator for repressing transgene expression during large scale production in Tet repressor expressing HEK293 lines. Multi-antigen expression strategies include two different 2A sequences.

Adenovirus-735 (PSCA-F2A-PSMA-mIRES-PSA).

Ad-735 is the viral equivalent of plasmid 459. Expression of the three antigens is driven off a single CMV promoter with a tetracycline operator for repressing transgene expression during large scale production in Tet repressor express-

ing HEK293 lines. Multi-antigen expression strategies include a 2A sequence and an IRES.

Adenovirus-796 (CBA-PSA, CMV-PSCA-pIRES-PSMA).

Ad-796 is the viral equivalent of plasmid 846. Expression of PSA is driven off the chicken beta actin promoter while PSCA and PSMA expression is driven off the CMV-TetO2 promoter. Multi-antigen expression strategies include two promoters and an IRES.

Adenovirus-809 (CBA-PSA, CMV-PSCA-F2A-PSMA).

Ad-809 is the viral equivalent of plasmid 850. Expression of PSA is driven off the chicken beta actin promoter while PSCA and PSMA expression is driven off the CMV-TetO2 promoter. Multi-antigen expression strategies include two promoters and a 2A sequence.

Example 5

Immunogenicity of Triple Antigen DNA Vaccines

Example 5 illustrates the capability of triple antigen nucleic acid vaccine constructs expressing PSMA, PSCA and PSA to elicit antigen-specific T and B cell responses to all three encoded prostate antigens.

Cellular Immune Response Study.

Immunogenicity of triple antigen constructs containing PSMA, PSCA and PSA, as described in Example 5, was studied in C57BL/6 mice according to the procedure described below.

Female C57BL/6 mice were primed on day 0 and boosted on days 14, 28 and 49 with DNA vaccine constructs encoding human-PSMA, PSCA and PSA antigens by PMED administration. In total, four different triple antigen vaccination strategies were evaluated, which included three DNA vaccines that co-expressed the target proteins and one co-formulation approach. For co-expression, single DNA plasmids encoding all three prostate antigens linked by 2A peptides or internal ribosome entry sites (IRES) were used as follows: PSA-F2A-PSMA-T2A-PSCA (plasmid ID#457), PSA-T2A-PSCA-F2A-PSMA (plasmid ID#458) and PSCA-F2A-PSMA-IRES-PSA (plasmid ID#459). For the co-formulation approach, three different DNA plasmids, each individually encoding PSMA, PSCA or PSA, were co-

formulated onto a single gold particle for PMED delivery. With the exception of co-formulation, the DNA elements that control co-expression (2A and IRES) differ in length, transgene expression efficiency and the presence of foreign genetic material attached to the target transgenes. As controls, C57BL/6 mice were vaccinated with DNA expressing a single prostate antigen, either PSMA, PSCA or PSA. For the co-expressed triple or single antigen DNA vaccines, a dose 2 µg of DNA vaccine plasmid was given per PMED administration, whereas 1 µg of each of the co-formulated triple antigen DNA vaccines (a total of 3 µg) was administered per PMED administration. Cellular immune responses against the triple and single antigen vaccines were measured by collecting the spleens from each animal on day 56, seven days after the final PMED vaccination. Splenocytes were isolated and subjected to an IFN- γ ELISPOT assay to measure the PSMA, PSCA and PSA-specific T cell responses. Briefly, 2 \times 10⁵ splenocytes from individual animals were plated per well with 5 \times 10⁴ per well of TRAMP-C2 (transgenic adenocarcinoma mouse prostate) cells stably expressing a single human prostate antigen or PSMA, PSCA and PSA together, or with individual or pools of human PSMA, PSCA and PSA-specific peptides at 10 µg/ml (see Table 22 for peptides and peptide pool composition), or medium alone as a control. Each condition was performed in triplicate. The plates were incubated for 20 h at 37° C. and 5% CO₂, washed and developed after incubation as per the manufacturer's instructions. The number of IFN- γ spot forming cells (SFC) was counted by a Cellular Technology Ltd. (CTL) reader. The results are presented in FIGS. 9 and 10, which show the average number of PSMA, PSCA or PSA-specific SFCs+/-the standard deviation of five mice per group, normalized to 1 \times 10⁶ splenocytes.

TABLE 22

The 15mer PSMA, PSCA and PSA peptides that were tested in the ELISPOT assay. The amino acid position of the N and C-terminal end of each peptide is indicated.

Prostate antigen	Peptides	Tested individually or pool
PSMA	577-591	Individual
PSMA	589-603	Individual
PSMA	601-615	Individual
PSMA	629-643	Individual
PSMA	641-655	Individual
PSMA	77-91	Pool 1
	91-111	
	153-167	
	229-243	
	365-379	
PSMA	401-415	Pool 2
	429-443	
	521-535	
	613-627	
PSMA	657-671	Pool 3
	685-699	
	701-715	
	733-747	
PSCA	25-39	Individual
PSA	65-79	Individual
PSA	73-87	Individual

Antibody Response Study.

Antibody responses against the triple and single antigen vaccines were measured by collecting the serum from each animal on day 56, seven days after the final PMED vaccination. Serum was subjected to enzyme-linked immunosorbent assays (ELISA) to determine the anti-PSMA and anti-PSCA antibody titers. In brief, ELISA plates were coated with 1 µg/ml of human PSMA or PSCA and incubated

overnight at 4° C. Plates were then blocked and incubated at RT for 1 h with 1% bovine serum albumin (BSA). Each serum sample was serially diluted in duplicate starting at a 1:100 dilution and incubated for 1 h at RT. After washing, a horseradish-peroxidase (HRP)-conjugated goat anti-mouse polyclonal IgG antibody was incubated at RT for 1 h. After washing, the TMB Peroxidase EIA-Substrate was incubated at RT for 30 min. The colorimetric reaction was stopped by addition 1N sulfuric acid and the absorbance then read at 450 nm. Titration curves were plotted for each serum sample (sample dilution versus absorbance). The serum titer (subsequently transformed into reciprocal titer) was then taken as the most dilute serum sample tested with an optical density (OD) value of above the lower limit of detection (LLOD; background plus 3 standard deviations) or the serum dilution calculated to achieve an OD value of 1.0. The results are presented in FIGS. 11 and 12, which show the average titers+/-the standard deviation of five mice per group.

Serum was also subjected to a fluorescence-activated cell sorting (FACS) assay to measure antibody binding to either human PSMA or PSCA expressed on the cell surface of appropriate cell lines, thus determining whether antibodies generated by the multi-antigen vaccines were capable of recognizing native PSMA and PSCA conformations, respectively. LNCaP (human prostate adenocarcinoma) cells were utilized to measure antibody binding to native PSMA. PC3 (human prostate cancer) cells served as a control in the FACS assay, as these cells do not express human PSMA. MIA-PaCa-2 (human pancreatic carcinoma) cells transduced with an adenovirus expressing human PSCA (Ad-PSCA) were utilized to measure antibody binding to native PSCA. Untransduced MIA-PaCa-2 cells served as the control. In brief, to measure anti-PSMA antibody binding, 2 \times 10⁵ LNCaP or PC3 cells were incubated with a 1:100 dilution of mouse serum or 15 µg/ml of the control mouse anti-human PSMA monoclonal antibody (mAb) (clone J591-A) for 20 min at 4° C. To measure anti-PSCA antibody binding, 2 \times 10⁵ Ad-PSCA transduced and untransduced MIA-PaCa-2 cells were incubated with a 1:30 dilution of mouse serum or 4 µg/ml of the control mouse anti-human PSCA mAb (clone 7F5) for 20 min at 4° C. Subsequently, cells were washed and incubated with a secondary Phycoerythrin (PE)-conjugated goat anti-mouse IgG antibody and a live/dead dye for an additional 20 min at 4° C. After the incubation, cells were washed and resuspended in 1.5% paraformaldehyde, and 10,000 live cells were acquired on a FACS Canto II. The results are presented in FIGS. 13 and 14, which show the average fold change in mean fluorescence intensity (MFI) of the mouse serum over the secondary anti-mouse antibody alone+/-the standard deviation of five mice per group. Antibody titers were not measured because PSA was expressed as a cytoplasmic protein by the multi-antigen vaccines investigated in this study.

Results:

FIGS. 9A-9D show the results of a representative study that evaluates the cellular immune responses of the triple antigen vaccines by IFN- γ ELISPOT assay. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, seven days after the last PMED vaccination, recognition of the endogenous prostate antigens was assessed by examining T cell responses to (A) TRAMP C2-PSMA, (B) TRAMP C2-PSCA, (C) TRAMP C2-PSA, and (D) TRAMP C2-PSMA-PSA-PSCA cells by IFN- γ ELISPOT assay. The TRAMP C2 cells served as a background control for the assay. For IFN- γ T cell responses to endogenous PSMA, a significant response to TRAMP

73

C2-PSMA was observed following the single PSMA PMED vaccination, which was consistent with responses seen in other studies. Similar PSMA-specific IFN- γ T cell response to TRAMP C2-PSMA was detected following the triple antigen vaccinations. In contrast, complete ablation of the response was observed following co-formulated PSMA, PSCA and PSA vaccination (* indicates p<0.05 by two-way ANOVA). For IFN- γ T cell responses to endogenous PSCA, no significant difference in response to the TRAMP C2-PSCA cells was observed when comparing the single PSCA vaccine to the four different triple antigen vaccines. For IFN- γ T cell responses to endogenous PSA, a significant decrease in the response magnitude to TRAMP C2-PSA was detected when comparing the immunogenicity of the single PSA vaccine to either PSCA-F2A-PSMA-IRES-PSA (** indicates p<0.001 by two-way ANOVA) or the co-formulated vaccine (* indicates p<0.05 by two-way ANOVA). When examining the response to TRAMP C2-PSMA-PSCA-PSA, the highest magnitude IFN- γ T cell response was observed following the PSA-F2A-PSMA-T2A-PSCA vaccine. Taken together, these data demonstrate recognition of endogenous PSMA, PSCA and PSA and generation of antigen-specific T cell responses to all three prostate antigens using a co-expression DNA vaccination strategy, especially with the PSA-F2A-PSMA-T2A-PSCA vaccine construct. However, the co-formulation DNA vaccination strategy resulted in a loss of antigen-specific IFN- γ T cell responses to PSMA and PSA.

FIGS. 10A-10D show the results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by IFN- γ ELISPOT assay. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, T cell responses to (A) PSMA peptides, (B) PSMA peptide pools, (C) PSCA peptides and (D) PSA peptides (see Table 22) were assessed by IFN- γ ELISPOT assay. Medium alone served as a background control for the assay. For IFN- γ T cell responses to both the individual and pools of PSMA peptides, compared to the single PSMA vaccine, the highest magnitude response was observed following administration of the PSA-F2A-PSMA-T2A-PSCA triple antigen vaccine. Similarly, the highest magnitude IFN- γ T cell response to PSCA and PSA-specific peptides was detected following administration of the PSA-F2A-PSMA-T2A-PSCA vaccine. The co-formulated PSMA, PSCA and PSA vaccine resulted in low to no T cell responses to the PSMA-specific peptides and low magnitude responses to the PSCA and PSA-specific peptides. These data also demonstrate generation of T cell responses to PSMA, PSCA and PSA when co-expressed from the same vaccine construct. There was consistent and robust IFN- γ T cell responses to all three prostate antigens following PSA-F2A-PSMA-T2A-PSCA vaccination, and significant decreases in the magnitude of IFN- γ T cell responses to the prostate antigens following PSA-T2A-PSCA-F2A-PSMA, PSMA-F2A-PSMA-IRES-PSA and co-formulated PSMA, PSCA and PSA vaccinations.

FIG. 11 shows the results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSMA antibody titers. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, serum anti-PSMA antibody titers were assessed by ELISA. All animals vaccinated with PSMA generated significant anti-PSMA antibody titers. There were no significant differences between titers, although vaccination with PSA-F2A-PSMA-T2A-PSCA resulted in slightly lower titers compared to the other groups vaccinated with PSMA. These data demonstrate the generation of anti-

74

PSMA-specific antibodies following triple antigen vaccination, using both co-expression and co-formulation vaccine strategies.

FIG. 12 shows the results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSCA antibody titers. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, serum anti-PSCA antibody titers were assessed by ELISA. Antibody titers were detected in mice vaccinated with PSCA alone and co-formulated PSMA, PSCA and PSA. These results indicate that co-formulation of PSMA, PSCA and PSA elicits a detectable anti-PSCA antibody titer compared to the co-expressed DNA vaccination strategies.

FIG. 13 shows the results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSMA antibody cell-surface binding. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, recognition of cell-surface native PSMA was assessed by serum antibody binding to LNCaP and PC3 cells. The PC3 cells served as a background control for the assay. PSA-F2A-PSMA-T2A-PSCA vaccination resulted in anti-PSMA antibodies with a significantly lower binding capacity to LNCaP cells compared to mice vaccinated with PSA-T2A-PSCA-F2A-PSMA and PSMA alone (* indicates p-value <0.05 by one-way ANOVA). All other PSMA vaccinated groups showed no significant difference in anti-PSMA antibody binding. The fold-change over secondary antibody alone for the J591-A mAb was 45.3 (data not shown). Overall, these data demonstrate generation of anti-PSMA-specific antibodies that recognize native PSMA following triple antigen vaccination, using both co-expression and co-formulation DNA vaccination strategies.

FIG. 14 shows the results of a representative study that evaluates the immunogenicity of the triple antigen vaccines by anti-PSCA antibody cell-surface binding. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, recognition of cell-surface native PSCA was assessed by serum antibody binding to Ad-PSCA transduced and untransduced MIA-PaCa-2 cells. The untransduced, parental cells served as a background control for the assay. With the exception of the single PSMA and single PSA cyto vaccines, all vaccine regimens with PSCA resulted in significant anti-PSCA antibody binding to Ad-PSCA transduced MIA-PaCa-2 cells compared to the parental cells. There were no significant differences in the anti-PSCA antibody binding to Ad-PSCA transduced MIA-PaCa-2 cells between the PSCA-vaccinated groups (one-way ANOVA, p-value >0.05). The fold change over secondary antibody alone for the 7F5 mAb was 18.7 (data not shown). Overall, these data demonstrate the generation of anti-PSCA-specific antibodies that recognize native PSCA following triple antigen vaccination, using both co-expression and co-formulation DNA vaccination strategies.

Example 6

Immunogenicity of Dual Antigen Vaccines

The following examples are provided to illustrate the capability of dual antigen vaccines expressing two prostate antigens to elicit antigen-specific T and B cell responses to the two encoded prostate antigens.

6A. Immunogenicity of Dual Antigen Vaccines Containing PSMA and PSCA in C57BL/6:

Study Procedure.

Cellular Immune Response Study.

Female C57BL/6 mice were primed on day 0 and boosted on days 14, 28, 42 and 70 with human PSMA and PSCA

expressing DNA by PMED epidermal injection. In total, five different dual antigen DNA vaccination strategies were evaluated, which included four DNA vaccines that co-expressed the antigens and one co-formulation approach. For co-expression, single DNA vaccine plasmids encoding two prostate antigens, PSMA and PSCA, linked by a dual promoter, 2A peptides or IRES were administered. These included PSMA-PSCA dual promoter (plasmid ID#460), PSMA-T2A-PSCA (plasmid ID#451), PSCA-F2A-PSMA (plasmid ID#454) and PSCA-IRES-PSMA (plasmid ID#603). For co-formulation, two different DNA plasmids, each individually encoding PSMA and PSCA, were co-formulated onto a single gold particle for PMED delivery. With the exception of co-formulation, the DNA elements that control co-expression (dual promoter, 2A and IRES) differ in length, transgene expression efficiency and the presence of foreign genetic material attached to the target transgenes. As controls, C57BL/6 mice were vaccinated with DNA expressing a single prostate antigen, PSMA or PSCA. For the co-expressed dual or single antigen DNA vaccines, a total dose of 2 µg of DNA vaccine was given per PMED administration, whereas 2 µg of each DNA vaccine plasmid (total of 4 µg of DNA per administration) was given for the co-formulation. Cellular immune responses of the dual and single antigen vaccines were measured by collecting the spleens from each animal on day 77, seven days after the final PMED vaccination. Splenocytes were isolated and subjected to an IFN-γ ELISPOT assay to measure the PSMA and PSCA-specific T cell responses. Briefly, 2×10^5 splenocytes from individual animals were plated per well with 5×10^4 per well of TRAMP-C2 cells expressing a single endogenous human prostate antigen or PSMA, PSCA and PSA together, or with individual or pools of human PSMA and PSCA-specific peptides at 10 µg/ml (see Table 22 for peptides and peptide pool composition), or medium alone as a control. Each condition was performed in triplicate. The plates were incubated for 20 h at 37° C. and 5% CO₂, washed and developed after incubation as per the manufacturer's instructions. The number of IFN-γ SFC was counted by a CTL reader. The results are presented in FIGS. 15 and 16, which show the average number of PSMA or PSCA-specific SFCs+/- the standard deviation of five mice per group, normalized to 1×10^6 splenocytes.

Antibody Response Study.

Antibody responses against the dual and single antigen vaccines were measured by collecting the serum from each animal on day 77, seven days after the final PMED vaccination. The anti-PSMA and anti-PSCA antibody titers in the serum was determined using ELISA as described in Example 5. The results are presented in FIGS. 17 and 18, which show the average titers+/- the standard deviation of five mice per group.

Serum was also subjected to a FACS assay to measure antibody binding to either human PSMA or PSCA expressed on the cell surface of appropriate cell lines, thus determining whether antibodies generated by the multi-antigen vaccines were capable of recognizing native PSMA and PSCA conformations, respectively. Antibody binding to cell-surface native PSA was not measured because PSA was expressed as a cytoplasmic protein by the multi-antigen vaccines investigated in this study. The FACS assay was conducted according to procedure as described in Example 5. The results presented in FIGS. 19 and 20, show the average fold-change in MFI of the mouse serum over the secondary anti-mouse antibody alone+/- the standard deviation of five mice per group. Antibody titers and binding to cell-surface native

PSA were not measured because PSA was expressed as a cytoplasmic protein by the multi-antigen vaccines investigated in this study.

Results.

FIGS. 15A-15C show the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by IFN-γ ELISPOT assay. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28, 42 and 70. On day 77, recognition of endogenous PSMA and PSCA was assessed by examining T cell responses to (A) TRAMP C2-PSMA, (B) TRAMP C2-PSCA and (C) TRAMP C2-PSMA-PSA-PSCA cells by IFN-γ ELISPOT assay. The TRAMP C2 cells served as a background control for the assay. For IFN-γ T cell responses to endogenous PSMA, the magnitude of the response TRAMP C2-PSMA was significantly decreased following vaccination with PSMA-PSCA dual promoter, PSMA-T2A-PSCA, PSCA-IRES-PSMA and co-formulated PSMA PSCA compared to vaccination with PSMA alone (** and *** indicate p-values <0.01 and <0.001, respectively, by two-way ANOVA). However, the PSCA-F2A-PSMA vaccine construct elicited a similar magnitude IFN-γ T cell response to the TRAMP C2-PSMA cells as the single PSMA vaccine. For IFN-γ T cell responses to endogenous PSCA, significantly increased responses were observed following vaccination with several of the dual antigen vaccines, including PSMA-PSCA dual promoter, PSCA-F2A-PSMA, PSCA-IRES-PSMA and co-formulated PSMA PSCA compared to the PSCA vaccine alone (*, ** and *** indicate p-values of <0.05, 0.01 and 0.001, respectively, by two-way ANOVA). The PSCA-T2A-PSMA vaccine construct elicited a similar magnitude IFN-γ T cell response to the TRAMP C2-PSCA cells as the single PSCA vaccine. Comparing the IFN-γ T cell responses to TRAMP C2-PSMA-PSA-PSCA, there were no significant differences between the groups vaccinated with different dual antigen vaccines. Taken together, these data demonstrate generation of PSMA and PSCA-specific T cell responses following dual antigen vaccination, using both co-expression and co-formulation DNA vaccination strategies.

FIGS. 16A-16C show the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by IFN-γ ELISPOT assay. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28, 42 and 70. On day 77, T cell responses to (A) PSMA peptides, (B) PSMA peptide pools and (C) PSCA peptides (see Table 22) were assessed by IFN-γ ELISPOT assay. Medium alone served as a background control for the assay. For IFN-γ T cell responses to both the individual and pools of PSMA peptides, the highest magnitude responses compared to the single PSMA vaccine were observed following the PSMA-T2A-PSCA and PSCA-F2A-PSMA dual antigen vaccinations. A significant reduction in the IFN-γ T cell response to the individual PSMA peptides was observed following vaccination with PSMA-PSCA dual promoter, PSCA-IRES-PSMA and co-formulated PSMA PSCA. The IFN-γ T cell response to the PSCA-specific peptide was similar between the groups vaccinated with the different dual antigen vaccines. These data also demonstrate generation of T cell responses to both PSMA and PSCA when co-expressed on the same DNA vaccine construct, or delivered as a co-formulation.

FIG. 17 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody titers. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28, 42 and 70. On day 77, serum anti-PSMA antibody titers

were assessed by ELISA. All animals vaccinated with PSMA generated significant anti-PSMA antibody titers. Mice vaccinated with the dual vaccine construct, PSCA-F2A-PSMA, and the single PSMA vaccine generated significantly higher antibody titers compared to all other groups of mice vaccinated with PSMA (one-way ANOVA, p-value <0.05). Vaccination with PSMA-PSCA dual promoter and co-formulated PSMA and PSCA resulted in higher antibody titers compared to mice that received the PSMA-T2A-PSCA vaccine. Taken together, these data demonstrate generation of anti-PSMA-specific antibodies following dual antigen DNA vaccination with PSMA and PSCA, using both co-expression and co-formulation vaccination strategies.

FIG. 18 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody titers. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28, 42 and 70. On day 77, serum anti-PSCA antibody titers were assessed by ELISA. Mice vaccinated with the co-formulated PSMA and PSCA, and the single PSCA vaccine generated significantly higher antibody titers compared to all other groups of mice vaccinated with PSCA (one-way ANOVA). Vaccination with PSMA-PSCA dual promoter resulted in higher antibody titers compared to vaccination with PSMA-T2A-PSCA, PSCA-F2A-PSMA and PSCA-IRES-PSMA. These results indicate that co-expression or co-formulation of PSMA and PSCA elicits anti-PSCA antibodies.

FIG. 19 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody cell-surface binding. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28, 42 and 70. On day 77, recognition of cell-surface native PSMA was assessed by serum antibody binding to LNCaP and PC3 cells. The PC3 cells served as a background control for the assay. With the exception of the single PSCA vaccine, all vaccine regimens with PSMA resulted in significant anti-PSMA antibody binding to LNCaP cells compared to the control PC3 cells. There were no significant differences in the anti-PSMA antibody binding to LNCaP cells between the PSMA-vaccinated groups (one-way ANOVA, p-value >0.05). The fold change over secondary antibody alone for the J591-A mAb was 45.3 (data not shown). These data demonstrate generation of anti-PSMA-specific antibodies that recognized native PSMA following dual antigen DNA vaccination, using both co-expression and co-formulation vaccination strategies.

FIG. 20 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody cell-surface binding. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28, 42 and 70. On day 77, recognition of cell-surface native PSCA was assessed by serum antibody binding to Ad-PSCA transduced and untransduced MIA-PaCa-2 cells. The untransduced, parental cells served as a background control for the assay. With the exception of the single PSMA vaccine, all vaccine regimens with PSCA resulted in significant anti-PSCA antibody binding to Ad-PSCA transduced MIA-PaCa-2 cells compared to the control cells. There were no significant differences in the anti-PSCA antibody binding to Ad-PSCA transduced MIA-PaCa-2 cells between the PSCA-vaccinated groups (one-way ANOVA, p-value >0.05). The fold change over secondary antibody alone for the 7F5 mAb was 18.7 (data not shown). Overall, these data demonstrate generation of anti-PSCA-specific antibodies that recognized native PSCA following dual antigen DNA vaccination, using both co-expression and co-formulation vaccination strategies.

6B. Immunogenicity of Dual Antigen Vaccines Containing Either PSMA and PSA or PSCA and PSA in C57BL/6 Study Procedure.

Cellular Immune Response Study.

5 Female C57BL/6 mice were primed on day 0 and boosted on days 14 and 28 with human PSMA, PSCA and PSA expressing DNA by PMED epidermal injection. In total, four different dual antigen vaccines strategies were evaluated, which included two co-expression approaches and two 10 co-formulation strategies. For co-expression, a single DNA plasmid encoding two prostate antigens, PSMA and PSA linked a 2A peptide (plasmid ID#5300) or PSCA and PSA linked by IRES (plasmid ID#455) were administered. For 15 co-formulation, plasmids individually encoding PSMA, PSCA or PSA were co-formulated onto a single gold particle for PMED delivery. Specifically, these included PSMA and PSA co-formulated and PSCA and PSA co-formulated. As controls, C57BL/6 mice were vaccinated with DNA expressing a single prostate antigen, PSMA, PSCA or PSA. For the 20 co-expressed dual or single antigen vaccines, a dose 2 µg of DNA was given per PMED administration, whereas 2 µg of each DNA vaccine plasmid (total of 4 µg of DNA per administration) was given for the co-formulation. Cellular 25 immune responses of the dual and single antigen vaccines were measured by collecting the spleens from each animal on day 35. Splenocytes were isolated and subjected to an IFN- γ ELISPOT assay to measure the PSMA, PSCA and PSA-specific T cell responses. Briefly, 2×10^5 splenocytes from individual animals were plated per well with 5×10^4 per 30 well of TRAMP-C2 cells expressing a single endogenous human prostate antigen or PSMA, PSCA and PSA together, or with individual or pools of human PSMA, PSCA and PSA-specific peptides at 10 µg/ml (see Table 22 for peptides and peptide pool composition), or medium alone as a 35 control. Each condition was performed in triplicate. The plates were incubated for 20 h at 37° C. and 5% CO₂, washed and developed after incubation as per manufacturer's instructions. The number of IFN- γ SFC was counted by a CTL reader. The results are presented in FIGS. 21 and 22, 40 which show the average number of PSMA, PSCA and PSA-specific SFCs+/-the standard deviation of five mice per group, normalized to 1×10^6 splenocytes.

Antibody Response Study.

Female C57BL/6 mice were primed on day 0 and boosted 45 on days 14, 28 and 49 with human PSMA, PSCA and PSA expressing DNA by PMED. Antibody responses against the dual and single antigen vaccines were measured by collecting the serum from each animal on day 56, seven days after the final PMED vaccination. The anti-PSMA and anti-PSCA antibody titers in the serum was determined using ELISA assay as described in Example 5. The results are presented in FIGS. 23 and 24, which show the average titers+/-the standard deviation of five mice per group.

Serum was also subjected to a FACS assay to measure 55 antibody binding to either human PSMA or PSCA expressed on the cell surface of appropriate cell lines, thus determining whether antibodies generated by the multi-antigen vaccines were capable of recognizing native PSMA and PSCA conformations, respectively. Antibody binding to cell-surface native PSA was not measured because PSA was expressed as a cytoplasmic protein by the multi-antigen vaccines investigated in this study. The FACS assay was conducted according to the procedure as described in Example 5. The results are presented in FIGS. 25 and 26, which show the average 60 fold change in MFI of the mouse serum over the secondary anti-mouse antibody alone+/-the standard deviation of five mice per group. Antibody titers and binding to cell-surface 65

native PSA were not measured because PSA was expressed as a cytoplasmic protein by the multi-antigen vaccines investigated in this study.

Results.

FIGS. 21A-21D show the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by IFN- γ ELISPOT assay. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14 and 28. On day 35, recognition of endogenous PSMA, PSCA and PSA was assessed by examining T cell responses to (A) TRAMP C2-PSMA, (B) TRAMP C2-PSCA, (C) TRAMP C2-PSA and (D) TRAMP C2-PSMA-PSA-PSCA cells by IFN- γ ELISPOT assay. The TRAMP C2 cells served as a background control for the assay. For IFN- γ T cell responses to endogenously expressed PSMA on cells, no significant differences were observed between responses to TRAMP C2-PSMA following vaccination with dual antigens containing PSMA (PSA-F2A-PSMA and co-formulated PSMA and PSA) and PSMA alone. Likewise, for IFN- γ T cell responses to endogenous PSCA, there were no observed differences in response magnitude to TRAMP C2-PSCA between the dual PSCA-IRES-PSA and co-formulated PSCA and PSA vaccines compared to the single PSCA vaccine. For IFN- γ T cell responses to endogenous PSA, a significant increase in the response magnitude to TRAMP C2-PSA was detected when comparing the immunogenicity of the single PSA vaccine to either PSA-F2A-PSMA (** indicates p<0.001 by two-way ANOVA) and co-formulated PSMA and PSA (** indicates p<0.01 by two-way ANOVA). There were no observed differences in response magnitude to TRAMP C2-PSA when comparing animals that received the dual PSCA and PSA vaccines to the single PSA vaccine. When examining the IFN- γ T cell response to TRAMP C2-PSMA-PSA-PSCA, there were no significant differences in the response between the groups vaccinated with different dual antigen vaccines. Taken together, these data demonstrate the generation of PSMA and PSA-specific T cell responses, as well as PSCA and PSA-specific T cell responses following dual antigen DNA vaccination, using both co-expression and co-formulation vaccine strategies.

FIG. 22 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody titers. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, serum anti-PSMA antibody titers were assessed by ELISA. All animals vaccinated with PSMA generated significant anti-PSMA antibody titers. There was no significant difference in the antibody titers between mice vaccinated with PSA-F2A-PSMA, co-formulated PSMA and PSA, and PSMA alone (one-way ANOVA, p-value >0.05). Taken together, these data demonstrate the generation of anti-PSMA-specific antibodies following dual antigen DNA vaccination with PSMA and PSA, using both co-expression and co-formulation vaccine strategies.

FIG. 23 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody titers. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, serum anti-PSCA antibody titers were assessed by ELISA. All animals vaccinated with PSCA generated significant anti-PSCA antibody titers. There was no significant difference in the antibody titers between mice vaccinated with PSCA-IRES-PSA, co-formulated PSCA and PSA, and PSCA alone (one-way ANOVA, p-value >0.05), although the antibody titers generated following PSCA-IRES-PSA vaccination trended lower than the other

groups vaccinated with PSCA. These results indicate that co-expression or co-formulation of PSCA and PSA elicits anti-PSCA antibodies.

FIG. 24 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSMA antibody cell-surface binding. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, recognition of cell-surface native PSMA was assessed by serum antibody binding to LNCaP and PC3 cells. The PC3 cells served as a background control for the assay. There were no significant differences in the anti-PSMA antibody binding to LNCaP cells between the PSMA-vaccinated groups (one-way ANOVA, p-value >0.05). The fold change over secondary antibody alone for the J591-A mAb was 45.3 (data not shown). Overall, these data demonstrate the generation of anti-PSMA-specific antibodies that recognized native PSMA following dual antigen vaccination, using both co-expression and co-formulation vaccine strategies.

FIG. 25 shows the results of a representative study that evaluates the immunogenicity of the dual antigen vaccines by anti-PSCA antibody cell-surface binding. Briefly, 5 mice per group were primed on day 0 and boosted with PMED on days 14, 28 and 49. On day 56, recognition of cell-surface native PSCA was assessed by serum antibody binding to Ad-PSCA transduced and untransduced MIA-PaCa-2 cells. The untransduced, parental cells served as a background control for the assay. All groups of mice vaccinated with PSCA demonstrated very low anti-PSCA antibody binding to Ad-PSCA transduced MIA-PaCa-2 cells. PSCA-IRES-PSA vaccination resulted in significantly decreased binding to Ad-PSCA transduced MIA-PaCa-2 cells compared to mice vaccinated with PSCA alone (* indicates p<0.05 by one-way ANOVA). Taken together, these data demonstrate that co-expression or co-formulation of PSCA and PSA results in very low recognition of native PSCA by anti-PSCA-specific antibodies.

Example 7

Immunogenicity of the Human Psma Modified Antigen

Study Design.

The immune responses induced by DNA vaccination using a construct encoding an immunogenic PSMA polypeptide (the “human PSMA modified antigen” or “hPSMA modified”) consisting 15-750 amino acids (aa) of the native human PSMA protein of SEQ ID NO: 1 were compared with those induced by the native human full-length PSMA protein (hPSMA full length). Groups of female C57BL/6 mice or female Pasteur (HLA-A2/DR1) transgenic mice were primed on day 0 and boosted on days 14, 28 by PMED administration with a 2 μ g dose of a DNA vaccine encoding either hPSMA full-length or hPSMA modified protein. Mice were bled and sacrificed on day 35 (7 days after the third vaccination) and T cell immune responses against the hPSMA full-length protein were determined in splenocytes by IFN- γ ELISPOT assay. For C57BL/6 mice, single cell suspensions of 5×10^5 splenocytes from individual animals were plated per well with 10 μ g purified hPSMA protein, 5×10^4 TRAMP-C2 cells alone, or TRAMP-C2 cells expressing hPSMA or a PSMA-PSA-PSCA fusion protein. For Pasteur (HLA-A2/DR1) transgenic mice, single cell suspensions of 5×10^5 splenocytes from individual animals were plated per well with 5×10^4 K562 cells expressing human HLA-A2 that had been pulsed with known HLA-A2-re-

stricted CD8⁺ T cell epitopes derived from the human PSMA protein sequence (Table 23). Responses in Pasteur mice were also determined using 10 µg/ml purified PSMA protein or 5×10⁴ SK-Mel5 cells that had been transduced with Adenoviral vectors expressing a control protein (Ad-eGFP) or the full-length human PSMA protein (Ad-hPSMA). Each condition was performed in triplicate. The plates were incubated for 20 h at 37° C. and 5% CO₂, washed and developed after incubation as per the manufacturer's instruction. The number of IFN-γ SFC was counted by a CTL reader. The results are presented in FIGS. 19 and 20, which show the average number of PSMA-specific SFC/million splenocytes+/-the standard deviation per group.

ELISA Assay.

Antibody responses induced by the modified and full-length PSMA vaccines were measured in serum from each animal collected on day 35. Serum from was subjected to ELISA to determine the anti-PSMA antibody titers in the serum was determined using the ELISA assay as described in Example 5. The results are presented in FIG. 26, which shows the average titers+/-the standard deviation of the number of mice per group.

FACS Assay.

Serum was also subjected to a FACS assay to measure antibody binding to either human PSMA expressed on the cell surface of appropriate cell lines, thus determining whether antibodies generated by the modified and full-length PSMA vaccines were capable of recognizing native PSMA conformation. The FACS assay was conducted according to the procedure as described in Example 5. The results are presented in FIG. 29, which show the average fold change in MFI of the mouse serum over the secondary anti-mouse antibody alone+/-the standard deviation of the number of mice per group.

TABLE 23

HLA-A2 restricted peptide epitopes tested in the assays conducted for the Pasteur (HLA-A2/DR1) transgenic mice. Peptides were tested individually at a concentration of 10 µg/ml. The amino acid position of the N and C-terminal end of each peptide is indicated.

Prostate antigen	Peptides	Purpose
hHer2	106-114	HLA-A2-restricted control peptide derived from the human Her2 protein
PSMA	168-177	HLA-A2-restricted PSMA test peptide
PSMA	663-671	HLA-A2-restricted PSMA test peptide
PSMA	275-289	HLA-A2-restricted PSMA test peptide

Results.

FIG. 26 shows the results of a representative study to evaluate the T cell immune response elicited by the human PSMA modified (aa 15-750) versus full-length human PSMA (aa 1-750) in C57BL/6 mice determined by IFN-γ ELISPOT assay. Five (5) mice per group were primed on day 0 and boosted PMED with DNA vaccines expressing hPSMA modified or hPSMA full-length proteins on days 14 and 28. On day 35, the response elicited against the hPSMA full-length protein were compared by determining T cell responses to TRAMP C2-PSMA or purified human PSMA ECD protein (referred to Purified hPSMA protein in FIG. 26) by IFN γ ELISPOT assay. TRAMP C2 cells served as a background control for the assay. The magnitude of the IFN-γ T cell responses elicited to TRAMP C2-PSMA or purified hPSMA protein were not significantly different (two-way ANOVA, p-value >0.05) between groups. These results indicate that the DNA vaccines expressing hPSMA

modified and hPSMA full-length proteins elicit equivalent T cell immune responses in C57BL/6 mice.

FIGS. 27A and 27B show the results of a representative study to evaluate the T cell immune response of human PSMA modified antigen (aa 15-750) versus full-length human PSMA antigen (aa 1-750) in Pasteur (HLA-A2/DR1) transgenic mice by IFN-γ ELISPOT assay. Ten (10) mice per group were primed on day 0 and boosted PMED with DNA vaccines encoding hPSMA modified or hPSMA full-length protein on days 14 and 28. On day 35, the T cell response elicited against the hPSMA full-length protein was determined by IFN-ELISPOT assay using (A) PSMA derived HLA-A2-restricted peptides representing known CD8⁺ epitopes and (B) SK-Mel5 cells transduced with Ad-hPSMA or purified hPSMA full-length protein. The hHER2 106 peptide and SK-Mel5 Ad-eGFP served as negative controls in the assays. The hPSMA modified vaccine elicited the highest magnitude of IFN-γ T cell immune responses to the HLA-A2-restricted CD8⁺ T cell epitopes, although the difference between groups was not significant (two-way ANOVA, p-value >0.05). Similarly, the hPSMA modified vaccine elicited the highest magnitude of immune response against the SK-Mel5 cells transduced with Ad-hPSMA and significantly (two-way ANOVA, p-value >0.05) higher frequencies of IFN-γ SFC to the purified hPSMA protein. These results demonstrate that the DNA vaccine expressing the hPSMA modified protein is more potent in inducing T cell responses to the hPSMA protein than the hPSMA full-length protein in Pasteur (HLA-A2/DR1) transgenic mice.

FIG. 28 shows the results of a representative study that evaluates the immunogenicity of the human modified and full-length PSMA vaccines by anti-PSMA antibody titers. Briefly, mice were primed on day 0 and boosted with PMED on days 14 and 28. Nine Pasteur mice were vaccinated with modified PSMA, 10 Pasteur mice were vaccinated with full-length PSMA, and 5 C57BL/6 mice per group were vaccinated with either modified or full-length PSMA. On day 35, serum anti-PSMA antibody titers were assessed by ELISA. As expected, C57BL/6 mice generated significantly greater anti-PSMA antibody titers compared to Pasteur mice (one-way ANOVA). Comparing antibody titers between the same strains of mice, there was no significant difference in the antibody titers between mice vaccinated with modified and full-length PSMA (one-way ANOVA, p-value >0.05). Overall, these results demonstrate that vaccination with the full-length version of human PSMA generates an equivalent anti-PSMA antibody titer compared to the human modified PSMA vaccine.

FIG. 29 shows the results of a representative study that evaluates the immunogenicity of the human modified and full-length PSMA vaccines by anti-PSMA antibody cell-surface binding. Briefly, 5 C57BL/6 mice per group were primed on day 0 and boosted with PMED on days 14 and 28. On day 35, recognition of cell-surface native PSMA was assessed by serum antibody binding to LNCaP and PC3 cells. The PC3 cells served as a background control for the assay. There were no significant differences in the anti-PSMA antibody binding to LNCaP cells between mice vaccinated with modified or full-length PSMA (one-way ANOVA, p-value >0.05). The fold change over secondary antibody alone for the J591-A mAb was 14.3 (data not shown). Overall, these data demonstrate that it is feasible to generate anti-PSMA-specific antibodies that recognized native PSMA following either modified or full-length PSMA vaccination.

83

Example 8

Effect of Anti-CTLA-4 Antibody on Vaccine-Induced Immune Response

The effect of local administration of anti-CTLA-4 monoclonal antibody (CP-675, 206) on the immune responses induced by a human PSMA nucleic acid molecule provided by the invention was investigated in a monkey study, in which the immune response was assessed by measuring PSMA specific T cell responses using an IFN γ ELISPOT assay.

Animal Treatment and Sample Collection.

Three groups of male Indian rhesus macaques, five to six (#1 to 5 or 6) per each test group, were immunized with a nucleic acid (SEQ ID NO: 10) that encodes a human PSMA modified antigen (SEQ ID NO: 9) delivered by adenovirus (1e11 V.P. injected intramuscularly) followed by 2 DNA immunizations (8 actuations/immunization, 4 actuations per each right and left side of the lower abdomen) by PMED with 6 and 9 week intervals respectively. Animals in Groups 2 and 3 additionally received bilateral intradermal injections of 3 mg of CpG (PF-03512676) subsequently after the PMED immunization in proximity to each inguinal draining lymph node. Group 2 also received intravenous injections of anti-CTLA-4 monoclonal antibody (CP-675, 206) at 10 mg/kg and group 3 received intradermal injections of anti-CTLA-4 monoclonal antibody (CP-675, 206) at 5 mg/kg in proximity to each left and right inguinal vaccine draining lymph node at the time of the second PMED immunization.

Peripheral blood samples were collected from each animal sixteen days after the last PMED immunization. Peripheral blood mononuclear cells (PBMCs) were isolated from the samples and were subjected to an IFN γ ELISPOT assay to measure the PSMA specific T cell responses. Briefly, 4e5 PBMCs from individual animals were plated per well with pools of PSMA specific peptides each at 2 ug/ml hPSMA ECD protein at 10 ug/ml, rhesus PSMA ECD protein at 10 ug/ml or nonspecific control peptides (human HER2 peptide pool) each at 2 ug/ml in IFN γ ELISPOT plates. The composition of each of the PSMA specific peptide pools is provided in Table 24A. The plates were incubated for 16 hrs at 37° C. and 5% CO₂ and washed and developed after incubation as per manufacturer's instruction. The number of IFN γ spot forming cells (SFC) were counted by CTL reader. Each condition was performed in duplicates. The results are

84

presented in Table 24B, which shows the average number of the PSMA specific SFC from the triplicates subtracting the average number of SFC from the nonspecific control peptides normalized to 1e6 PBMCs. A indicates that the count is not accurate because the numbers of spots were too numerous to count.

IFN γ ELISPOT Assay Procedure.

A capture antibody specific to IFN γ (BD Bioscience, #51-2525kc) is coated onto a polyvinylidene fluoride (PVDF) membrane in a microplate overnight at 4° C. The plate is blocked with serum/protein to prevent nonspecific binding to the antibody. After blocking, effector cells (such as splenocytes isolated from immunized mice or PBMCs isolated from rhesus macaques) and targets (such as PSMA peptides from peptide library, target cells pulsed with antigen specific peptides or tumor cells expressing the relevant antigens) are added to the wells and incubated overnight at 37° C. in a 5% CO₂ incubator. Cytokine secreted by effector cells are captured by the coating antibody on the surface of the PVDF membrane. After removing the cells and culture media, 100 μ l of a biotinylated polyclonal anti-humanIFN γ antibody was added to each of the wells for detection. The spots are visualized by adding streptavidin-horseradish peroxidase and the precipitate substrate, 3-amino-9-ethylcarbazole (AEC), to yield a red color spot as per manufacturer's (Mabtech) protocol. Each spot represents a single cytokine producing T cell.

Results.

Table 24B. shows the results of a representative IFN γ ELISPOT assay that evaluates and compares the T cell responses induced by the vaccine without (group 1) or with anti-CTLA-4 monoclonal antibody (CP-675, 206) given either systemically by intravenous injections (group 2) or locally by intradermal injections in proximity to the vaccine draining lymph node (group 3). As shown in Table 1B, PSMA vaccine induced measurable IFN γ T cell responses to multiple PSMA specific peptides and proteins in the absence of CpG (PF-03512676) and anti-CTLA-4 monoclonal antibody (CP-675, 206). The responses were modestly enhanced by the addition of CpG (PF-03512676) and systemic delivery of the anti-CTLA-4 antibody (CP-675, 206; group 2). However, a more potent and significant enhancement of the response to multiple PSMA peptides and PSMA protein was observed when the anti-CTLA-4 monoclonal antibody (CP-675, 206) was delivered locally by intradermal injections in proximity to the vaccine draining lymph node (group 3).

TABLE 24A

PSMA peptide pools: Each peptide pool (i.e., P1, P2, P3, H1, H2, R1, and R2) is composed of 15mer peptides from either human PSMA protein (hPSMA protein) or rhesus PSMA protein (rPSMA protein) sequences as indicated below. The amino acid position of the N and C-terminal end of each peptide is indicated.

P1	P2	P3	H1	H2	R1	R2
h 1-15	h 249-263	h 449-463	h 33-47	h 465-479	r 33-47	r 465-479
h 5-19	h 253-267	h 453-467	h 37-51	h 469-483	r 37-51	r 469-483
h 9-23	h 257-271	h 457-471	h 41-55	h 473-487	r 41-55	r 473-487
h 13-27	h 261-275	h 485-499	h 45-59	h 477-491	r 45-59	r 477-491
h 17-31	h 265-279	h 489-503	h 61-75	h 481-495	r 61-75	r 481-495
h 21-35	h 269-283	h 493-507	h 65-79	h 537-551	r 65-79	r 537-551
h 25-39	h 273-287	h 497-511	h 69-83	h 541-555	r 69-83	r 541-555
h 29-43	h 277-291	h 501-515	h 73-87	h 545-559	r 73-87	r 545-559
h 49-63	h 281-295	h 505-519	h 97-111	h 577-591	r 97-111	r 577-591
h 53-67	h 285-299	h 509-523	h 101-115	h 581-595	r 101-115	r 581-595
h 57-71	h 289-303	h 513-527	h 105-119	h 585-599	r 105-119	r 585-599
h 77-91	h 293-307	h 517-531	h 109-123	h 589-603	r 109-123	r 589-603
h 81-95	h 297-311	h 521-535	h 137-151	h 601-615	r 137-151	r 601-615
h 85-99	h 317-331	h 525-539	h 141-155	h 605-619	r 141-155	r 605-619
h 89-103	h 321-335	h 529-543	h 145-159	h 609-623	r 145-159	r 609-623

TABLE 24A-continued

PSMA peptide pools: Each peptide pool (i.e., P1, P2, P3, H1, H2, R1, and R2) is composed of 15mer peptides from either human PSMA protein (hPSMA protein) or rhesus PSMA protein (rPSMA protein) sequences as indicated below. The amino acid position of the N and C-terminal end of each peptide is indicated.

P1	P2	P3	H1	H2	R1	R2
h 93-107	h 325-339	h 533-547	h 149-163	h 613-627	r 149-163	r 613-627
h 113-127	h 329-343	h 549-563	h 209-223	h 637-651	r 209-223	r 637-651
h 117-131	h 333-347	h 553-567	h 213-227	h 641-655	r 213-227	r 641-655
h 121-135	h 353-367	h 557-571	h 217-231	h 645-659	r 217-231	r 645-659
h 125-139	h 357-371	h 561-575	h 221-235	h 649-663	r 221-235	r 649-663
h 129-143	h 361-375	h 565-579	h 301-315	h 653-667	r 301-315	r 653-667
h 133-147	h 365-379	h 569-583	h 305-319	h 657-671	r 305-319	r 657-671
h 153-167	h 369-383	h 573-587	h 309-323	h 709-723	r 309-323	r 709-723
h 157-171	h 373-387	h 593-607	h 313-327	h 713-727	r 313-327	r 713-727
h 161-175	h 377-391	h 597-611	h 337-351	h 717-731	r 337-351	r 717-731
h 165-179	h 381-395	h 617-631	h 341-355	h 721-735	r 341-355	r 721-735
h 169-183	h 385-399	h 621-635	h 345-359	h 725-739	r 345-359	r 725-739
h 173-187	h 389-403	h 625-639	h 349-363	h 729-743	r 349-363	r 729-743
h 177-191	h 393-407	h 629-643	h 461-475	h 733-747	r 461-475	r 733-747
h 181-195	h 397-411	h 633-647				
h 185-199	h 401-415	h 661-675				
h 189-203	h 405-419	h 665-679				
h 193-207	h 409-423	h 669-683				
h 197-211	h 413-427	h 673-687				
h 201-215	h 417-431	h 677-691				
h 205-219	h 421-435	h 681-695				
h 225-239	h 425-439	h 685-699				
h 229-243	h 429-443	h 689-703				
h 233-247	h 433-447	h 693-707				
h 237-251	h 437-451	h 697-711				
h 241-255	h 441-455	h 701-715				
h 245-259	h 445-459	h 705-719				
		h 737-750				

TABLE 24B

T cell responses induced by the vaccine without (Group 1) or with anti-CTLA-4 antibody Tremelimumab (CP-675, 206) given systemically by intravenous injections (Group 2) or local intradermal injections (Group 3).

Group	animal ID	recall antigen									
		P1	P2	P3	H1	H2	hPSMA protein	R1	R2	rPSMA protein	
1. no immune modulator	#1	7.5	62.5	0.0	210.0	172.5	455.0	3.8	1.3	0.0	
	#2	11.3	48.8	0.0	17.5	146.3	111.3	0.0	3.8	0.0	
	#3	12.5	342.5	13.8	115.0	517.5	705.0	12.5	40.0	138.8	
	#4	6.3	23.8	0.0	211.3	38.8	45.0	5.0	7.5	7.5	
	#5	0.0	16.3	0.0	0.0	52.5	45.0	0.0	0.0	0.0	
	#6	6.3	442.5	21.3	42.5	238.8	736.3	11.3	8.8	93.8	
2. with aCTLA4 (IV)	#1	23.8	57.5	1.3	71.3	292.5	278.8	6.3	6.3	0.0	
	#2	0.0	61.3	0.0	2.5	108.8	78.8	0.0	6.3	3.8	
	#3	58.8	41.3	7.5	1063.8	82.5	1197.5	22.5	7.5	1.3	
	#4	25.0	318.8	27.5	147.5	983.8	1046.3	26.3	86.3	2.5	
	#5	15.0	312.5	5.0	402.5	573.8	707.5	97.5	25.0	20.0	
3. with aCTLA4 (ID)	#1	48.8	1236.3	38.8	405.0	1236.3	1236.8	218.8	490.0	1120.0	
	#2	113.8	946.3	17.5	293.8	1247.5	1247.5	162.5	86.3	5.0	
	#3	16.3	1248.8	6.3	465.0	1248.8	1248.8	187.5	295.0	11.3	
	#4	6.3	828.8	6.3	1006.3	1247.5	1247.5	142.5	30.0	17.5	
	#5	152.5	566.3	18.8	757.5	1173.8	1242.5	287.5	57.5	110.0	

Example 9

Systemic Exposure of CTLA-4 Antibody after Administration in Monkeys

The blood levels of anti-CTLA-4 antibody Tremelimumab (CP675206) were investigated in Indian Rhesus macaques after the antibody was administered by intradermal or intravenous injections.

Animal Treatment and Sample Collection.

Three animals per treatment group were injected with the anti-CTLA-4 antibody Tremelimumab at 10 mg/kg, either 60 with a single intravenous injection into the saphenous vein or multiple 0.2 ml intradermal bilateral injections in the upper thigh in proximity to the inguinal draining lymph nodes. Blood samples were collected at 0, 1, 2, 4, 8, 12, 24, and 48 hrs post injection into 2.0 ml vaccutainer tubes containing lithium heparin as the anticoagulant. Plasma was collected from the supernatant in the vaccutainer tubes after centrifugation at 1500×g at 4° C. for 10 min. The levels of 65

Tremelimumab in the plasma was measured by a quantitative ELISA assay according to the procedure provided below.

Tremelimumab Quantitative ELISA Assay Procedure.

The 384-well high bind assay plates (VWR-Greiner Bio-One Cat#82051-264) were coated with 25 μ L/well of CD-152 (CTLA-4; Ancell Immunology Research Products Cat#501-020) at 1.0 μ g/ml in 100 mM carbonate-bicarbonate coating buffer and incubated overnight at 4° C. Plates were washed $\times 6$ with 1×PBS-Tween (0.01M PBS pH 7.4/0.05% Tween 20) and blocked using 40 μ L/well of 5% FBS/1×PBS-Tween and incubated shaking at 600 rpm RT for 1 hour. Standards were prepared by making the following dilutions of Tremelimumab: 200, 67, 22, 7.4, 2.5, 0.82, 0.27, 0.09 and 0.03 ng/mL. The samples were diluted to 1:100, 1:1,000 and 1:10,000. The diluent consisted of 1% naive cynomolgus macaque sera and 5% FBS in 1×PBS-Tween (0.01M PBS pH 7.4/0.05% Tween 20). 25 μ L/well of each standard, sample and diluent control were transferred in duplicate into the plate and incubated shaking at 600 rpm RT for 1 hour. After washing $\times 6$ with 1×PBS-Tween, 25 μ L/well of secondary antibody (goat anti-human IgG HRP, Southern Biotech Cat#9042-05) at a 1:5,000 dilution with 1×PBS-Tween was added and then incubated shaking at 600 rpm room temperature for 1 hour. After washing $\times 6$ with 1×PBS-Tween, 25 μ L/well of TMB Peroxidase EIA-Substrate (solution A+B) (Bio-Rad Cat#172-1067) were added and the plates were incubated at RT for 4 minutes. The colorimetric reaction was stopped by addition of 12.5 μ L/well 1N Sulfuric acid and the absorbance then read at 450 nm. The amount of Tremelimumab in each sample was quantified using the standard curve with 0.27 to 67 ng/mL used as the quantitative range.

Results.

The plasma anti-CTLA-4 levels from a representative study are presented in FIG. 30. As shown, intradermal injection of the anti-CTLA-4 antibody Tremelimumab displays a slower release kinetics of the antibody in the blood and a lower systemic exposure ($AUC_{0-24}=4.9\times 10^6$ ng·hr/ml) profile than intravenous injections ($AUC_{0-24}=7.2\times 10^6$ ng·hr/ml).

Example 10

Effect of Anti-CTLA-4 Antibody on Vaccine-Induced Immune Responses in Mice

Study Procedure.

Female BALB/c mice, 6 per group, were primed and boosted with rHer-2 expressing DNA by PMED separated by a four week interval. 150 μ g of the monoclonal antibody specific to mouse CTLA-4 (clone 9H10, Bioxcell or #BE0131) or isotype control monoclonal antibodies (Bioxcell #BE0091) was administered on the days of PMED actuation and 100 μ g on the days after PMED by local intradermal or systemic intraperitoneal injections as indicated in the legends. The polyfunctional (multi-cytokine positive) T cell immune responses were measured from splenocytes isolated from individual mice 7 days after the last PMED immunizations by ICS assay. After a 5 hr stimulation with a vaccine specific epitope peptide (rHer-2 specific antigen specific CD8 (p66), CD4 (p169) epitope or irrelevant peptide HBV (core antigen p87)) at 10 μ g/ml, the splenocytes were first stained for CD4, CD3 and CD8 which was followed by permeabilization and staining for IFN α , TNF α and IL-2 expression that was analyzed by flow cytometry. The total number of antigen specific single,

double or triple cytokine positive T cells per total spleen of each animal is calculated by subtracting the responses to the irrelevant peptide HBV from the vaccine specific responses and normalized by the total number of splenocytes isolated per spleen.

Results.

FIGS. 31A and 31B show the results of a representative study that evaluates the immunomodulatory activity of anti-CTLA-4 monoclonal antibody (clone 9H10) on the quality of the vaccine induced immune responses by intracellular cytokine staining assay. Seven days after the last PMED, significant increases in antigen specific single and double cytokine positive CD8 T cell responses by the local intradermal delivery of anti-CTLA-4 and double and triple cytokine positive CD8 T cell responses by the systemic delivery was observed. Additionally, significant increases in antigen specific single cytokine positive CD4 T cells by intradermal delivery and double cytokine positive cells by systemic delivery of anti-CTLA-4 was observed (*indicates $P<0.05$ by Student's T-test).

Example 11

Synergistic Effect of Sunitinib in Combination with an Anti-Cancer Vaccine

Study Procedure.

The Anti-tumor efficacy of sunitinib malate in combination with an anti-cancer DNA vaccine was investigated in 30 BALB/neuT transgenic female mice. Heterozygote BALB/neuT transgenic female mice that express rat Her-2 (rHer-2) tumor associate antigen were implanted subcutaneously with 1e6 TUBO cells expressing rHer-2 which are derived from the spontaneous mammary tumors of BALB/neuT mice. After 7 days post tumor cell implantation, the mice were dosed once a day orally with either vehicle or sunitinib malate at doses as indicated in the legends. Three days after the initiation of sunitinib malate therapy, the mice were immunized with regimens comprised of either (a) control vaccine that expresses an antigen that is not expressed in the tumor or the mouse or (b) DNA cancer vaccine construct that expresses a rat Her-2 antigen of SEQ ID NO: 54 (rHER2) which is expressed in the tumor and the mouse. The tumor growth rate was analyzed by measuring the long (a) and short diameter (b) of the subcutaneous TUBO tumors twice a week and calculating the volume as $a\times b^2\times 0.5$ mm 3 . The average and standard error of the mean of the tumor volumes from 10 mice per each treatment group are plotted against the days after tumor implantation.

Results.

FIG. 32 shows the results of a representative study that evaluates and compares the subcutaneous tumor growth rate upon treatment with sunitinib malate as a monotherapy or in combination with the DNA cancer vaccine. While the tumors from mice that received the DNA cancer vaccine (rHER2: intramuscular injection of 1e9 V.P. of rHer-2 expressing adenovirus followed by two biweekly actuations of rHer-2 expressing DNA by PMED) continued to grow rapidly, the tumors from mice that received sunitinib malate at either 20 mg/kg or 40 mg/kg doses with control vaccines (control: intramuscular injection of 1e9 V.P. of eGFP expressing adenovirus followed by two biweekly actuations of HBV core antigen expressing DNA by PMED) significantly decreased the tumor growth rate, with 20 mg/kg displaying suboptimal efficacy compared to the 40 mg/kg dose. However when the cancer vaccine was co-administered with the suboptimal dose of sunitinib malate at 20

89

mg/kg, the tumors grew at a much slower growth rate than in mice treated with the same dose of sunitinib malate co-administered with a control vaccine and similar to that of mice treated with sunitinib malate at a higher dose. Cancer vaccine provides additional therapeutic benefit to mice that received suboptimal doses of sunitinib malate.

FIGS. 33A-33D show the individual tumor growth rates of mice from a representative study that evaluates and compares the anti-tumor efficacy of sunitinib malate at 20 mg/kg with control (control) or the DNA cancer vaccine (rHER2). Briefly, after 7 days post tumor cell implantation, ten mice per treatment group were daily orally dosed with either vehicle or 20 mg/kg sunitinib malate (Sutent) for 34 days. Three days after the initiation of Sutent dosing, a series of immunizations, primed by Adenovirus followed by PMED, were initiated that continued after the discontinuation of Sutent therapy. Specifically the mice were immunized with either control vaccine comprised of an intramuscular injection of 1e9 V.P. of eGFP expressing adenovirus subsequently followed by two biweekly, two 9 days and four weekly actuations of DNA expressing HBV core and surface antigens by PMED or cancer vaccine comprised of rHer-2 expressing adenovirus and DNA instead. The tumors of the animals that received vehicle with the control vaccine became measurable around day 7 and continued growing reaching 2000 mm³ after 50 days post tumor implant. The tumor growth of the animals that received Sutent with control vaccine was significantly impaired until Sutent therapy was discontinued. The tumors displayed a rapid growth rate immediately after the discontinuation of Sutent, the majority reaching 2000 mm³ after 50 days post tumor implant. The tumor growth rate of animals that only received the cancer vaccinations was modestly slower than the animals that did not receive cancer vaccine or Sutent. The combination of cancer vaccine with Sutent not only suppressed the tumor growth during Sutent therapy (FIG. 33) but also significantly impaired the progression of the tumor in 60% of the animals after discontinuation of Sutent treatment.

FIG. 34 shows the Kaplan-Meier survival curve of the groups of mice from the study described in FIGS. 33A-33D FIG. 2B that evaluates the anti-tumor efficacy of Sutent with the control (control) or cancer vaccine (rHER2). The mice were sacrificed when the tumor volume reached 2000 mm³ according to IAUCUC guidelines. Only mice treated with Sutent (at 20 mg/kg) and cancer vaccine displayed a significantly prolonged survival compared to mice either treated with vehicle and control vaccine, cancer vaccine without Sutent or Sutent without cancer vaccine (*P<0.01 by Log-rank Test).

FIGS. 35A-35D show the percentage of myeloid derived suppressor cells (Gr1+CD11b+) and Treg containing CD25+ CD4+ cells in the periphery blood from the groups of mice from the study described in FIGS. 33A-33D. Briefly, PBMCs were stained and analyzed by flow cytometry for the expression of Gr1, CD11b, CD3, CD4, and CD25 from submandibular bleeds of five mice from each group on d27 (20 days post the initiation of Sutent or vehicle treatment). The mean and standard error of the mean of each treatment group is shown. A statistically significant reduction of % myeloid derived suppressor cells was observed in mice that were treated by Sutent with either control or cancer vaccine compared to mice that did not receive Sutent nor cancer vaccine (vehicle+control). However significantly lower myeloid derived suppressor cells were observed in mice treated with the combination of Sutent with cancer vaccine (Sutent+rHER2) compared to mice that were treated with

90

cancer vaccine without Sutent (vehicle+rHER2) or Sutent without cancer vaccine (Sutent+control). A statistically significant reduction of Treg containing CD25+CD4+ T cells in the CD4 population was observed by Sutent with cancer vaccine. These mice had significantly lower % of Treg containing CD25+CD4+ T cells in the CD4 population than mice that were treated with cancer vaccine without Sutent or Sutent without cancer vaccine in their blood. * indicates P<0.05 by Student's T-test.

FIGS. 36A-36C show the total number of myeloid derived suppressor cells (Gr1+CD11b+), Tregs (CD4+ CD25+Foxp3+) and PD-1+CD8 T cells isolated from tumors of mice. Briefly, the mice were given a single daily oral dose of either vehicle or Sutent at 20 mg/kg three days after implantation with TUBO cells for 28 days. The same mice were immunized with either control vaccine comprised of an intramuscular injection of 1e9 V.P. of eGFP expressing adenovirus subsequently followed by two biweekly administrations of DNA expressing HBV core antigen delivered by PMED or cancer vaccine comprised of rHer-2 expressing adenovirus and DNA. An intradermal injection of 50 µg of CpG (PF-03512676) was given with the PMED administrations in proximity to the right side inguinal draining lymph node. Seven days after the second PMED and CpG administration, individual tumors were isolated, from 6 mice per treatment group. The single cell suspension prepared from the isolated subcutaneous tumors were stained by antibodies specific for Gr1, Cd11 b, CD3, CD8, CD25, FoxP3, and PD-1 and analyzed by flow cytometry. The mean and standard error of the mean of the total number of specific cells as indicated in the figures per µg of tumor from each treatment group is plotted. (*indicates P<0.05 by Student's T-test) While there was no significant difference in the frequency of immune suppressive Tregs or MDSC found in the tumor when mice were given cancer vaccine (A and B) compared to mice that received control vaccine (A and B), a significant reduction was observed when the mice were treated with Sutent (A and B) compared to mice that received cancer vaccine only. A reduction of PD-1+CD8 T cells was also observed in mice that were treated with Sutent (C) compared to mice that received cancer vaccine (C) only. Taken together, these data demonstrate that agents that reduce Tregs, MDSCs or CD8+PD-1+Tcells in combination with the vaccine would be beneficial in reducing tumor burden in tumor bearing animals.

Example 12

Anti-Cancer Efficacy of Vaccine in Combination with Sunitinib and Anti-CTLA-4 Antibody

The anti-tumor efficacy of a cancer vaccine in combination with sunitinib and anti-CTLA-4 monoclonal antibody (clone 9D9) was investigated in subcutaneous TUBO tumor bearing BALB/neut mice.

Study Procedure.

Briefly, ten mice per each group were daily orally dosed with either vehicle or sunitinib malate at 20 mg/kg starting at day 10 post tumor implant until day 64. Vaccination with DNA constructs that either encode no antigen (control vaccine) or a rat Her-2 antigen of SEQ Id NO: 54 (cancer vaccine) as adenovirus vectors initiated on day 13 subsequently followed by two weekly immunizations, two biweekly immunizations, and seven weekly immunizations of respective antigens (HBV antigens or rHer-2) by DNA. The groups of mice (closed circle and open triangle) that were treated with anti-murine CTLA-4 monoclonal antibody

91

were intraperitoneally injected with 250 µg of the antibody on day 20, 27, 41, 55, 62, 69, 76, 83, 90, and 97 right after the PMED injections.

Results.

FIG. 37 shows the Kaplan-Meier survival curve of the groups of mice from a representative study evaluating the anti-tumor efficacy of sunitinib and anti-murine CTLA-4 monoclonal antibody (clone 9D9) in combination with a cancer vaccine. Increased survival time was observed in mice treated with Sutent with control vaccine (open circle), anti-murine CTLA-4 monoclonal antibody (open triangle) or cancer vaccine (closed triangle). A further increase of survival was observed in mice treated with Sutent and cancer vaccine in combination with anti-murine CTLA-4 (closed circle). P values were calculated by log-rank test.

Example 13

Systemic Exposure of Sunitinib and Anti-Cancer Efficacy of Anti-Cancer Vaccine in Combination with Low Dose Sunitinib

Sunitinib Systemic Exposure Study.

The kinetics of blood sunitinib was investigated in BALB/ neuT mice with subcutaneous TUBO tumors. Briefly, 20 mice per each treatment group were given Sutent orally, at 20 mg/kg once a day (SID) or at 10 mg/kg twice a day (BID) with 6 hr intervals, 6 days after tumor implantation. Submandibular blood from 2-3 mice was collected into lithium heparin tubes at several time points after Sutent dosing as indicated (0, 2, 4, 6, 8, 10, 12, and 24 hr). The plasma supernatant was recovered from the tubes after centrifugation at ×1000 g for 15 min. and the sunitinib levels from the plasma samples were measured by LC/MS/MS. The mean and standard error of the mean of each group at each time point is plotted.

Results are presented in FIG. 38. The mean and standard error of the mean of each group at each time point is plotted. The dotted horizontal line marks the minimum sunitinib blood level, 50 ng/ml, that is necessary to effectively inhibit tumor growth in monotherapy (Mendel, D., et al.: "In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship". Clinical Cancer Research, 203, 9:327-337). As shown, the blood sunitinib levels in the mice that received either 20 mg/kg SID or 10 mg/kg BID only maintain the target effective dose of 50 ng/ml that effectively inhibits tumor growth transiently within 24 hr. The blood levels in 20 mg/kg SID group peaked above 50 ng/ml at 2 hrs, dropped to 50 ng/ml at 6 hrs and cleared the blood by 12 hrs post Sutent dosing. The levels in the group that received 10 mg/kg BID peaked above 50 ng/ml at 2 hrs but rapidly dropped below 50 ng/ml by 4 hrs that peaked again 2 hrs after the second dose. The levels rapidly dropped to 50 ng/ml by 4 hrs and cleared the blood by 18 hrs after the second dose. Despite the bi-daily dosing regimen, the animals that received 10 mg/kg, remained to display lower duration of exposure at target concentration than the 20 mg/kg single daily dosing regimen.

Anti-Tumor Efficacy Study.

Anti-tumor efficacy of long term administration of low dose sunitinib in combination with an anti-cancer vaccine was investigated in BALB/neuT mice with subcutaneous TUBO tumors. Briefly, the mice were given sunitinib malate (Sutent) for 31 days at 20 mg/kg SID or for 104 days at 10 mg/kg BID and received either control vaccine or cancer

92

vaccine. The control vaccine, which delivers no antigen, and the cancer vaccine, which delivers a rat her-2 antigen (rHer-2) of SEQ ID NO: 54, was given by adenovirus on day 9 subsequently followed by five biweekly administrations of the DNA by PMED delivering HBV antigens or rHer-2 respectively.

The results are presented in FIG. 39. While the cancer vaccine improved the survival of mice given Sutent at 20 m/kg, there was even significant improvement of the survival of mice given Sutent at 10 mg/kg (*P=0.05 by Log rank test).

Example 14

Effect of CpG or CD40 Agonist on the Immune Responses Induced by Cancer Vaccine

Immunogenicity Studies in BALB/c Mice

The effect of local administration of immune modulators on the magnitude and quality of antigen specific immune responses induced by a cancer was investigated in BALB/c mice, in which the immune response was assessed by measuring rHER2 specific T cell responses using the IFN γ ELISPOT assay or intracellular cytokine staining assay. Briefly, 4 to 6 female BALB/c mice per group as indicated were immunized with DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) by PMED delivery system. The immune modulators, CpG7909 (PF-03512676) and anti-CD40 monoclonal agonistic antibody, were administered locally by intradermal injections in proximity to the vaccine draining inguinal lymph node subsequently after the PMED actuations. Antigen specific T cell responses were measured by IFN γ ELISPOT or intracellular cytokine staining assay according to the procedure described below.

Intracellular Cytokine Staining (ICS) Assay

The rHer-2 specific polyfunctional (multi-cytokine positive) T cell immune responses were measured from splenocytes or PBMCs isolated from individual animals by ICS assay. Typically 1e6 splenocytes were incubated with Brefeldin A at 1 µg/ml and peptide stimulant (rHer-2 specific CD8 p66, rHer-2 specific CD4 p169 or irrelevant HBV p87) at 10 µg/ml for 5 hr at 37° C. in a 5% CO₂ incubator. After the stimulation, the splenocytes were washed and blocked with Fcγ block (anti-mouse CD16/CD32) for 10 min. at 4° C. followed by a 20 min staining with Live/dead aqua stain, anti-mouse CD3ePE-Cy7, anti-mouse CD8a Pacific blue, and anti-mouse CD45R/B220 PerCP-Cy5.5. The cells were washed, fixed with 4% paraformaldehyde overnight at 4° C., permeabilized with BD fix/perm solution for 30 min at RT and incubated with anti-mouse IFN γ APC, anti-mouse TNF α Alexa488 and anti-mouse IL-2 PE for 30 min at RT. The cells were washed and 20,000 CD4 or CD8 T cells were acquired for analysis by flow cytometry. The total number of antigen specific single, double or triple cytokine positive T cells per total spleen of each animal is calculated by subtracting the rHer-2 specific responses to the irrelevant peptide HBV from the vaccine specific responses and normalized to the total number of splenocytes isolated from the spleen.

IFN γ ELISPOT Assay Results

FIG. 40 shows the IFN γ ELISPOT results from groups of mice from a representative study evaluating the magnitude of antigen specific T cell responses induced by the rHER2 vaccine when given with the immune modulators as indicated. Briefly, each mouse per treatment group (n=4) was immunized with DNA plasmid expression constructs encod-

93

ing rHER2 antigen sequences (SEQ ID NO:54) by PMED immediately followed by either 100 µg of control rat IgG monoclonal antibody (Bioxcell #BE0089: control mAb) or 50 µg CpG7909 or 100 µg of anti-CD40 monoclonal antibody (Bioxcell #BE0016-2: a-CD40 mAb) as indicated. The antigen specific immune responses were measured by IFN γ ELISPOT assay from 5e5 splenocytes mixed with control or rHer-2 specific p66 peptides at 10 µg/ml concentration, 7 days after the PMED actuation. The number of total IFN γ secreting cells from splenocytes containing 1e5 CD8 T cells were calculated from the ELISPOT results from individual animals and the % of CD8 T cells in splenocytes and mean and standard mean of error of each group are plotted. As shown, both CpG7909 and the anti-CD40 monoclonal antibody both significantly enhanced the magnitude of antigen specific immune responses induced by rHer-2 DNA compared to mice that received control antibodies.

Intracellular Cytokine Staining (ICS) Assay Results.

FIGS. 41A and 41B show the results of a representative study that evaluates the immunomodulatory activity of CpG 7909 on the quality of the vaccine induced immune responses by intracellular cytokine staining assay. Briefly, each animal was immunized twice with the DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) delivered by PMED with a 4-week interval. The mice in each group (n=5) were given intradermal injections of either PBS (PMED group) or 50 µg of CpG 7909 (PMED+CpG group) in proximity to the right side vaccine draining inguinal node immediately following both DNA immunizations by PMED. Seven days after the last immunization by PMED, an ICS assay was performed on the splenocytes isolated from each individual mice to detect antigen specific polyfunctional CD8 or CD4 T cells that secrete IFN γ , TNF α and/or IL-2. A significant increase in rHer-2 specific multi-cytokine positive CD8 and CD4 T cell responses were detected from mice treated with the local delivery of CpG 7909 compared to PBS. An increase in the single cytokine positive CD8 population was observed in the animals that received local delivery of CpG7909 administration compared to PBS (*indicates P<0.05 by Student's T-test).

FIGS. 42A and 42B show the results of a representative study that evaluates the immunomodulatory activity of an agonistic anti-CD40 monoclonal antibody on the quality of the vaccine induced immune responses by intracellular cytokine staining assay. Briefly, each animal was immunized twice by DNA plasmid expression constructs encoding rHER2 antigen sequences (SEQ ID NO:54) delivered by PMED with a 4 week interval. The mice in each group (n=6) were given 100 µg of intradermal injections of either isotype IgG control (PMED with IgG) or anti-CD40 monoclonal antibody (PMED with aCD40) in proximity to the right side vaccine draining inguinal node, one day after the first immunization was administered by PMED. Seven days after the last PMED, an ICS assay was performed on the splenocytes isolated from each individual mice to detect rHer-2 specific polyfunctional CD8 or CD4 T cells that secrete IFN γ , TNF α and/or IL-2. A significant increase in the rHer-2 specific triple-cytokine positive CD8 and CD4 T cell responses were detected from mice treated with the local delivery of anti-CD40 monoclonal antibody compared to isotype IgG control. There were also significant increases in rHer-2 specific single and double cytokine positive CD4 T cells by anti-CD40 monoclonal antibody given locally (*indicates P<0.05 by Student's T-test).

94

Example 15

Anti-Cancer Efficacy of Cancer Vaccine in Combination with Low Dose Sunitinib

Anti-tumor efficacy of anti-cancer vaccine in combination with low dose sunitinib was investigated in BALB/neuT mice with spontaneous mammary pad tumors.

Animal Treatment.

Briefly, 13-14 weeks old female mice were orally given sunitinib malate (Sutent) at 5 mg/kg for 112 days twice a day. The control vaccine, which delivers no antigen, and cancer vaccine which delivers a rat Her-2 antigen of SEQ ID NO: 54 (rHer-2), were given by adenovirus injections on day 3 as a prime followed by 7 biweekly administrations by PMED of DNA delivering HBV antigens (control vaccine) or rHer-2 (cancer vaccine) respectively. The survival end point was determined when all ten mammary pads became tumor positive or when the volume of any of the mammary tumors reach 2000 mm³. The results are presented in FIG. 43.

Results.

Compared to previously published pharmacokinetic profile of Sutent (Mendel, D., Laird, D., et al.: "In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship". Clinical Cancer Research, 203, 9:327-337) and previous data (FIG. 38), the C_{Max} of Sutent in mice dosed twice a day at 5 mg/kg is expected to be significantly lower than the minimum blood levels necessary to achieve efficient anti-tumor efficacy in mice and man. The data shows a quick and temporary improvement in the survival of the mice treated with low dose Sutent monotherapy. However when given with the cancer vaccine, a more persistent and significant improvement of survival was observed (P<0.0001 by Log rank test).

Example 16

Enhancement of Vaccine-Induced Immune Responses by Local Administration of CpG

The immune enhancement of local administration of CpG (PF-03512676) on the immune responses induced by a human PSMA nucleic acid provided by the invention was investigated in a monkey study, in which the immune response was assessed by measuring PSMA specific T cell responses using an IFN γ ELISPOT assay.

Animal Treatment and Sample Collection.

Six groups of Chinese cynomolgus macaques, six (#1 to 6) per each test group, were immunized with a plasmid DNA encoding the human PSMA modified antigen (amino acids 15-750 of SEQ ID NO:1) delivered by electroporation. Briefly, all animals received bilateral intramuscular injections of 5 mg of plasmid DNA followed by electroporation (DNA EP) on day 0. Subsequently right after the electroporation, group 2 received bilateral intramuscular injections of 2 mg of CpG mixed with 1 mg Alum in proximity to the DNA injection sites. Group 3 and 4 received bilateral intramuscular injections of 2 mg of CpG delivered without alum in proximity to the DNA injection sites either on day 0 or day 3, respectively. Group 5 received 2 mg of bilateral intradermal injections of CpG delivered in proximity to the vaccine draining inguinal nodes on day 3. Group 6 received bilateral injections of 200 µg of CpG mixed with the DNA solution which was co-electroporated into the muscle on day 0.

IFN γ ELISPOT Assay Procedure.

Peripheral blood samples were collected from each animal fifteen days after the DNA immunization. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples and were subjected to an IFN γ ELISPOT assay to measure the PSMA specific T cell responses. Briefly, 4e5 PBMCs from individual animals were plated per well with pools of PSMA specific peptides or nonspecific control peptides (human HER2 peptide pool) each at 2 μ g/ml in IFN γ ELISPOT plates. The composition of each of the PSMA specific peptide pool is provided in Table 1A. The plates were incubated for 16 hrs at 37° C. and 5% CO2 and washed and developed after incubation as per manufacturer's instruction. The number of IFN γ spot forming cells (SFC) were counted by CTL reader. Each condition was performed in duplicates. The result of a representative experiment is presented in Table 1B. The reported PSMA specific response is calculated by subtracting the average number of the SFC to the nonspecific control peptides (human HER2 peptide pool) from the average number of SFC to the PSMA peptide pools and normalized to the SFC observed with 1e6 PBMCs. ^ indicates that the count is not accurate because the numbers of spots were too numerous to count. ND indicates not determined.

Results.

Table 28 shows the result of a representative IFN γ ELISPOT assay that evaluates and compares the IFN γ T cell responses induced by the vaccine without (group 1) or with CpG (PF-03512676) given locally by intramuscular (groups 2, 3, 4, and 5) or intradermal injections (group 6). There results in Table 1B is plotted in FIG. 1. As shown in Table 1B and FIG. 1, the PSMA specific IFN γ T cell responses were detected to multiple PSMA specific peptide pools in the absence of CpG (PF-03512676) in all six animals (group 1). The total response to the PSMA peptides measured were modestly higher in a few animals that additionally received CpG (PF-03512676) either by intramuscular (group 4: 3/6) or intradermal (group 5: 2/6) injections 3 days after DNA electroporation. However, when CpG was delivered subsequently right after electroporation on the same day (groups 2 and 3), there were several animals that failed to produce high responses (group 2: 4/6 and group 3: 3/6) whether mixed or not mixed with Alum. However higher net responses were detected in 4/6 animals when a ten-fold lower dose of CpG was co-electroporated with the DNA solution into the muscle (group 6) with a statistically higher response ($P<0.05$) to peptide pools H1 and R1 compared to animals that did not receive CpG (group 1). The data shows that low dose of CpG can effectively enhance IFN γ T cell responses induced by a DNA vaccine when co-electroporated into the muscle.

TABLE 28

PSMA specific IFN γ T cell responses induced by the DNA vaccine without (Group 1) or with CpG (Groups 2, 3, 4, 5 and 6) is measured by IFN γ ELISPOT assay from PBMCs, 15 days after DNA electroporation

Group	Animal ID	Recall Antigen					
		P1	P2	P3	H1	H2	R1
1	#1	36	31	1	126	183	5
	#2	6	3	13	61	524	6
	#3	11	4	8	108	1049	3
	#4	10	0	13	20	151	13
	#5	8	6	11	39	469	14
	#6	26	5	0	145	356	8

TABLE 28-continued

PSMA specific IFN γ T cell responses induced by the DNA vaccine without (Group 1) or with CpG (Groups 2, 3, 4, 5 and 6) is measured by IFN γ ELISPOT assay from PBMCs, 15 days after DNA electroporation							
Group	Animal ID	Recall Antigen					
		P1	P2	P3	H1	H2	R1
10	#1	3	10	0	15	35	0
	#2	0	0	8	4	6	13
	#3	3	0	0	0	10	11
	#4	6	209	4	111	414	23
	#5	15	5	30	171	104	68
	#6	0	0	0	9	9	8
15	#1	14	19	8	123	1066	10
	#2	14	16	20	384	393	104
	#3	0	0	15	0	6	0
	#4	0	0	0	33	21	0
	#5	4	91	1	875	1235	233
	#6	0	0	0	0	3	0
20	#1	0	33	15	1025	1209	280
	#2	0	313	3	23	656	6
	#3	61	120	61	428	1190	143
	#4	0	0	8	599	870	34
	#5	0	1	8	19	226	10
	#6	111	55	39	231	613	121
25	#1	21	9	0	355	1131	73
	#2	0	0	0	118	233	0
	#3	0	0	0	18	129	0
	#4	0	28	78	68	294	58
	#5	25	0	28	329	1125	134
	#6	0	0	0	23	39	4
30	#1	0	0	13	650	1096	270
	#2	34	1	74	124	474	29
	#3	0	3	14	684	1074	126
	#4	8	9	0	136	321	49
	#5	13	23	35	ND	1235	333
	#6	0	0	0	421	1201	138

Example 17

Enhancement of Vaccine-Induced Immune Responses by Local Administration of Anti-CTLA-4 Antibody

The effect of low dose subcutaneous administration of anti-CTLA-4 monoclonal antibody (CP-675, 206) on the immune responses induced by a rhesus PSMA nucleic acid was investigated in a monkey study, in which the immune response was assessed by measuring PSMA specific T cell responses using an IFN γ ELISPOT assay. The rhesus PSMA nucleic acid used in the study has the sequence as set forth in SEQ ID NO: 56 and encodes an immunogenic PSMA polypeptide of SEQ ID NO: 55.

Animal Treatment and Sample Collection.

Five groups of male Indian rhesus macaques, seven (#1 to 7) per each test group, were immunized with an adenovirus encoding a rhesus PSMA modified polypeptide delivered by bilateral intramuscular injections (2x 5e10 V.P.). Immediately following the adenovirus injections, group 1 received vehicle, and groups 2 to 4 received bilateral subcutaneous injections of anti-CTLA-4 antibody (CP-675, 206) at doses 2x 25 mg, 2x 16.7 mg and 2x 8.4 mg respectively in proximity to the vaccine draining lymph node.

Nine days after the immunization, peripheral blood mononuclear cells (PBMCs) were isolated from each animal and were subjected to an IFN γ ELISPOT assay to measure the rhesus PSMA specific T cell responses. Briefly, 4e5 PBMCs from individual animals were plated per well with pools of rhesus PSMA specific peptides (P1, P2, P3 or R1+R2

defined in table 24A) or nonspecific control peptides (human HER2 peptide pool) each at 2 ug/ml in IFN γ ELISPOT plates. The plates were incubated for 16 hrs at 37° C. and 5% CO₂ and washed and developed after incubation as per manufacturer's instruction. The number of IFN γ spot forming cells (SFC) were counted by CTL reader. Each condition was performed in duplicates. The average of the duplicates from the background adjusted SFC of the rhesus PSMA specific peptide pools was normalized to the response in 1e6 PBMCs. The individual and sum responses to the peptide pools from each individual animal are presented in Table 29.

IFN γ ELISPOT Assay Procedure.

A capture antibody specific to IFN γ BD Bioscience, #51-2525kc) is coated onto a polyvinylidene fluoride (PVDF) membrane in a microplate overnight at 4° C. The plate is blocked with serum/protein to prevent nonspecific binding to the antibody. After blocking, effector cells (such as splenocytes isolated from immunized mice or PBMCs

Results.

Table 29. shows the results of a representative IFN γ ELISPOT assay that compares the T cell responses induced by the vaccine without (group 1) or with (groups 2-4) anti-CTLA-4 monoclonal antibody (CP-675, 206) given locally by subcutaneous injections in proximity to the vaccine draining lymph node. The vaccine generated an immune response (group 1) that was significantly enhanced by the local administration of the anti-CTLA-4 antibody (CP-675, 206) at a dose of 50 mg (group 2, P=0.001 by Student's T-test using underestimated values). The response was also significantly enhanced by low doses of anti-CTLA-4 antibody at 33.4 mg (group 3: P=0.004 by Student T-test using underestimated values) and 16.7 mg (group 4: P=0.05 by Student T-test) respectively. The data suggests that low doses of anti-CTLA-4 delivered by subcutaneous injection can significantly enhance the vaccine induced immune responses.

TABLE 29

IFN γ T cell responses induced by the vaccine without (Group 1) or with subcutaneous injections of anti-CTLA-4 antibody (CP-675, 206).							
Group	dose, mg	animal ID	peptide pool				
			aCTLA4	P1	P2	P3	R1 + R2
1	NA	1		21	0	0	108
		2		59	480	28	353
		3		133	29	359	305
		4		0	28	1	35
		5		41	6	30	99
		6		1	0	849	169
		7		0	0	0	23
2	50.0	1	^1105	704	^1116	^1116	^4041
		2	371	26	661	779	1837
		3	393	559	216	198	1366
		4	^1100	^1100	406	1078	3684
		5	778	325	554	419	2076
		6	^1079	^1079	844	^1079	4081
		7	423	103	535	398	1459
3	33.4	1	^425	^425	^425	^425	^1700
		2	^580	^580	^580	^580	2320
		3	TNTC	TNTC	TNTC	TNTC	TNTC
		4	321	778	370	409	1878
		5	331	466	311	446	1554
		6	545	121	^631	^1194	^2491
		7	446	299	^1078	^1060	^2883
4	16.7	1	^964	296	^964	^964	3188
		2	76	76	76	76	304
		3	^984	^984	^984	^984	^3936
		4	260	489	648	^1109	^2506
		5	119	45	28	140	332
		6	55	76	43	198	372
		7	146	726	141	400	1413

^ indicates that the count is underestimated due to the high spot numbers.
TNTC means too numerous to count.

isolated from rhesus macaques) and targets (such as PSMA peptides from peptide library, target cells pulsed with antigen specific peptides or tumor cells expressing the relevant antigens) are added to the wells and incubated overnight at 37° C. in a 5% CO₂ incubator. Cytokine secreted by effector cells are captured by the coating antibody on the surface of the PVDF membrane. After removing the cells and culture media, 100 μ l of a biotinylated polyclonal anti-human IFN γ antibody was added to each of the wells for detection. The spots are visualized by adding streptavidin-horseradish peroxidase and the precipitate substrate, 3-amino-9-ethylcarbazole (AEC), to yield a red color spot as per manufacturer's (Mabtech) protocol. Each spot represents a single cytokine producing T cell.

55

60

65

Example 18

Immunomodulation of Myeloid Derived Suppressor Cells by Low Dose Sunitinib

The following example is provided to illustrate the immunomodulatory effects of low dose sunitinib on Myeloid Derived Suppressor Cells (MDSC) in vivo, in a non-tumor mouse model.

Study Procedures.

To generate MDSC enriched splenocytes, TUBO cells (1×10^6) were implanted into the flanks of 5 BALB/neuT mice, and left for approx. 20-30 days until tumor volume reached between 1000-1500 mm³. Mice were then sacri-

99

ficed, spleens removed and the MDSC enriched splenocytes recovered. Splenocytes were labeled for 10 minutes with 5 μ M CFSE, washed with PBS and counted. Labeled cells were subsequently resuspended at 5×10^7 splenocytes/ml in PBS solution and adoptively transferred via an i.v. tail vein injection into naïve BALB/c recipient mice. Three days prior to adoptive transfer, the recipient mice began bi-daily dosing with vehicle or sunitinib malate (Sutent) at 5 mg/kg, 10 mg/kg and 20 mg/kg. Following adoptive transfer, recipient mice continued to receive bi-daily dosing of Vehicle or sunitinib for two further days, after which point the mice were sacrificed, spleens removed, splenocytes recovered and processed for phenotypic analysis.

Splenocytes were counted and resuspended at 5×10^6 cells/ml in FACS staining buffer (PBS, 0.2% (w/v) bovine serum albumin, and 0.02% (w/v) Sodium Azide). For flow cytometry staining of splenocytes, 2.5×10^6 cells were first incubated with anti-bodies to CD16/CD32, 10 minutes at 4° C., to block Fc receptors and minimize non-specific binding. Splenocytes were then stained for 20 minutes at 4° C. with appropriate fluorophore conjugated antibodies (Biologend) to murine cell surface markers. For T cells (anti-CD3 (Pacific Blue), clone 17A2) and for MDSC (anti-GR-1 (APC), clone RB6-8C5 and anti-CD11b (PerCp Cy5.5), clone M1/70). A live/dead stain was also included. Following antibody incubation, stained splenocytes were washed with 2 mls of FACS buffer, pelleted by centrifugation and resuspended in 0.2 ml of FACS buffer prior to data acquisition on a BD CANTO II flow cytometer. To monitor the effect of Sunitinib or Vehicle on the adoptively transferred MDSC survival, we calculated the percentage of CFSE+, CD3-, GR1+, CD11b+ in the live, singlet gate. We then determined the number of adoptively transferred MDSC per spleen by calculating what actual cell number the percentage represented of total splenocytes count. Data was analyzed by FloJo and Graph pad software.

Results.

The data presented in Table 31 represents the mean number of adoptively transferred CSFE+, CD3-, GR1+, CD11b+ cells recovered per spleen (n=7/group), 2 days post adoptive transfer, from mice bi-daily dosed with either Vehicle or 5 mg/kg, 10 mg/kg and 20 mg/kg Sunitinib. The data demonstrates that Sunitinib, dosed bi-daily, *in vivo*, has an immunomodulatory effect on MDSCs, even when dosed as low as 5 mg/kg, resulting in a statistically significant reduction in the numbers recovered when compared to the vehicle treated control group.

TABLE 31

Mean number of CFSE+, CD3-, GR1+, CD11b+ MDSCs recovered from the spleen, 7 mice per group, and the corresponding standard error. Statistical significance was determined by one-way ANOVA using the Dunnett's multiple comparison test, comparing the Sunitinib dosed groups against the 0 mg/kg (vehicle) group.

	Sunitinib Dose (mg/kg)			
	0 (Vehicle)	5	10	20
MDSC #/spleen	17470 +/- 2017	10980 +/- 1082	4207 +/- 338	4440 +/- 440
Mean +/- SEM				
Statistical significance,	NA	Yes	Yes	Yes
p < 0.5 [??]				

100

Example 19

Immunogenicity of Triple Antigen Adenovirus and DNA Constructs

5

The following example is provided to illustrate the capability of triple antigen vaccine constructs (either in the form of adenovirus vector or DNA plasmid) expressing three antigens PSMA, PSCA and PSA provided by the invention to elicit specific T cell responses to all three encoded antigens in nonhuman primates.

In Vivo Study Procedures.

The T cell immunogenicity of five adenovirus vectors each expressing three antigens (PSMA, PSCA and PSA; Ad-733, Ad-734, Ad-735, Ad-796 and Ad-809) provided by the invention were compared to the mix of three adenovirus vectors each only expressing a single antigen (PSMA, PSA or PSCA), 9 days post prime. The response to single adenovirus expressing a single antigen (groups 1-3) was evaluated to demonstrate the specificity. Briefly, Indian rhesus macaques (n=6 for groups 1 and 3, n=7 for group 2 and n=8 for groups 4-9) were intramuscularly injected with a total of $1e11$ V.P. followed by intradermal injections of anti-CTLA-4 at 10 mg/kg on the same day. Nine days after the injections, peripheral blood mononuclear cells (PBMCs) were isolated from each animal and were subjected to an IFN γ ELISPOT assay to measure the PSMA, PSA and PSCA specific T cell responses.

Thirteen weeks after the adenovirus and anti-CTLA-4 injections when the T cell responses have contracted, the monkeys received DNA (Group 1: PSMA, plasmid 5166; Group 2: PSA, plasmid 5297; Group 3: PSCA, plasmid 5259; Group 4: mix of PSMA, PSA and PSCA, plasmids 5166, 5259 and 5297; Group 4: plasmid 457; Group 6: plasmid 458; Group 7: plasmid 459; Group 8: plasmid 796 and Group 9: plasmid 809) boost vaccinations delivered by electroporation. In summary, each animal received a total 5 mg of plasmid DNA provided by the invention which delivers the same expression cassette encoded in the adenovirus used in the prime. Nine days after the boost vaccination, peripheral blood mononuclear cells (PBMCs) were isolated from each animal and were subjected to an IFN γ ELISPOT assay.

IFN γ ELISPOT Assay.

Briefly, 4e5 PBMCs from individual animals were plated per well with PSMA specific peptide pools P1, P2, P3 or H1 and H2 (Table 24A), PSA specific pool 1 or 2 (Table 25), PSCA specific pool (Table 26) or nonspecific control peptides (human HER2 peptide pool) each at 2 μ g/ml in IFN γ ELISPOT plates. The plates were incubated for 16 hrs at 37° C. and 5% CO₂ and washed and developed after incubation

101

as per manufacturer's instruction. The number of IFN γ spot forming cells (SFC) were counted by CTL reader. Each condition was performed in duplicates. The average of the duplicates from the background adjusted SFC of the antigen specific peptide pools was normalized to the response in 1e6 PBMCs. The antigen specific responses in the tables present the sum of the responses to the corresponding antigen specific peptides or peptide pools.

Results:

Table 27 represents a study that evaluates the T cell immunogenicity of five different adenoviruses each expressing all three antigens in comparison to the mixture of three adenoviruses each expressing a single antigen in Indian rhesus macaques by IFN γ ELISPOT. The majority of animals that only received Ad-PSMA (group 1) injections induced specific responses to PSMA but not to PSA or PSCA (Student's T-test, P<0.03. One animal (#4) that induced responses to PSCA preferentially was removed from the statistical analysis). The animals that only received injections of Ad-PSA (group 2) induced specific responses to PSA but not to PSMA or PSCA (Student's T-test, P<0.02). The animals that only received injections of Ad-PSCA (group 3) induced specific responses to PSCA but not to PSMA or PSA (Student's T-test, P<0.03). All five triple-antigen expressing adenovirus vectors (groups 5-9) induced IFN γ T cell responses to all three antigens which the magnitude varied by animal. The magnitude of the responses to PSCA induced by the triple antigen expressing adenoviruses were similar to the mix of individual vectors (group 4). However the magnitude of responses to PSMA induced by Ad-809 (group 9) and responses to PSA induced by Ad-796 (group 8) were each significantly superior to the mix (Student's T-test, P=0.04 and P=0.02) respectively. These results indicate that vaccinating with an adenovirus expressing triple antigens can elicit equivalent or superior T cell immune responses to vaccinating with the mix of individual adenoviruses in nonhuman primates.

Table 28 shows the IFN γ ELISPOT results represents a study that evaluates the immunogenicity of the five different triple antigen expression cassettes provided in the invention delivered by an adenovirus prime in combination with anti-CTLA-4 followed by an electroporation boost of the corresponding plasmid DNA. The immune responses are compared to the mix of three constructs expressing a single antigen delivered similarly by an adenovirus prime with anti-CTLA-4 and DNA electroporation boost immunizations.

All of the animals that only received Ad-PSMA with anti-CTLA-4 followed by plasmid-PSMA (group 1) immunizations induced specific responses to PSMA but not to PSA or PSCA. Similarly all of the animals that only received Ad-PSA with anti-CTLA-4 followed by plasmid-PSA immunizations (group 2) induced specific responses to PSA but not to PSMA or PSCA and finally all of the animals that only received Ad-PSCA with anti-CTLA-4 followed by plasmid-PSCA (group 3) immunizations induced specific responses to PSCA but not to PSMA or PSA (Student's T-test, P<0.01).

All animals that have been immunized with either the triple-antigen expressing vectors (groups 5-9) or the mix (group 4) induced IFN γ T cell responses to all three antigens. The frequency of PSCA or PSA specific IFN γ T cells detected were similar in all of these groups (groups 4-9) respectively. However construct groups 7 and 9 that received triple antigen expression vector vaccinations produced significantly higher frequency of responses to PSMA than the mix of three single antigen expressing constructs (group 4).

102

These results indicate that adenovirus and DNA vaccines expressing triple antigens in one cassette can elicit equivalent or superior IFN γ T cell responses to the mix of adenoviruses and DNAs expressing the single antigens in nonhuman primates.

TABLE 25

PSA peptide pools: The amino acid position and sequence of fifteen amino acid peptides overlapping by thirteen amino acids from PSA peptide library is shown.			
	PSA peptide pool 1		PSA peptide pool 2
amino acid no.	PSA peptide sequence	amino acid no.	PSA peptide sequence
5-19	VVFLTLSVTWIGAAP	129-143	PAAELTDAVKVMDLPT
9-23	TLSVTWIGAAPLILS	131-145	ELTDAVKVMDLPTQEQE
11-25	SVTWIGAAPLILSRI	133-147	TDAVKVMDLPTQEQEPAA
13-27	TWIGAAPLILSRIVG	135-149	AVKVMDLPTQEQPALGG
15-29	IGAAPLILSRIVGGW	137-151	KVMDLPTQEQPALGTT
17-31	AAPLILSRIVGGWECEK	139-153	MDLPTQEQPALGTTCTY
19-33	PLILSRIVGGWECEK	141-155	LPTQEQPALGTTCYAS
21-35	IILSRIVGGWECEKHQS	143-157	TQEQPALGTTCYASGW
23-37	SRIVGGECEKHQSQP	145-159	EPALGTTCYASGWGS
25-39	IVGGWECEKHQSQPWQ	147-161	ALGTTCYASGWGSIE
27-41	GGWECEKHQSQPWQL	149-163	GTTCYASGWGSIEPE
29-43	WECEKHQSQPWQLVVA	151-165	TCYASGWGSIEPEEF
31-45	CEKHQSQPWQLVASR	153-167	YASGWGSIEPEEFLT
33-47	KHSQPWQLVASRGR	155-169	SGWGSIEPEEFLTPK
35-49	SQPWQLVASRGRAV	157-171	WGSIEPEEFLTPKKL
37-51	PWQVLVASRGRAVCG	159-173	SIEPEEFLTPKKLQC
39-53	QVLVASRGRAVCGGV	161-175	EPEEFLTPKKLQCVD
41-55	LVASRGRAVCGGVLV	163-177	EEFLTPKKLQCVDLH
43-57	ASRGRAVCGGVLVHP	165-179	FLTPKKLQCVDLHV
45-59	RGRAVCGGVLVHPQW	167-181	TPKKLQCVDLHVISN
47-61	RAVCGGVLVHPQWVL	169-183	KKLQCVDLHVISNDV
49-63	VCGGVLVHPQWVLTA	171-185	LQCVDLHVISNDVCA
51-65	GGVLVHPQWVLTAHH	173-187	CVDLHVISNDVCAQV
53-67	VLVHPQWVLTAACI	175-189	DLHVISNDVCAQVHP
55-69	VHPQWVLTAACIRN	177-191	HVISNDVCAQVHPQK
57-71	PQWVLTAACIRNKS	179-193	ISNDVCAQVHPQKV
59-73	WVLTAACIRNKSIV	181-195	NDVCAQVHPQKVTKF
61-75	LTAACIRNKSIVLL	183-197	VCAQVHPQKVTKF
63-77	AAHCIRNKSIVLLGR	185-199	AQVHPQKVTKFMLCA
65-79	HCIRNKSIVLLGRHS	187-201	VHPQKVTKFMLCAGR
67-81	IRNKSIVLLGRHSLF	189-203	PQKVTKFMLCAGRWT
69-83	NKSIVLLGRHSLFHP	191-205	KVTKFMLCAGRWTGG

103

TABLE 25-continued

PSA peptide pool 1		PSA peptide pool 2	
amino acid no.	PSA peptide sequence	amino acid no.	PSA peptide sequence
71-85	SVILLGRHSLFHPED	193-207	TKFMLCAGRWTGGKGS
73-87	ILLGRHSLFHPEDTG	195-209	FMLCAGRWTGGKSTC
75-89	LGRHSLFHPEDTGQV	197-211	LCAGRWTGGKSTCSG
77-91	RHSLFHPEDTGQVFQ	199-213	AGRWTGGKSTCGDS
79-93	SLFHPEDTGQVFQVS	201-215	RWTGGKSTCGDGG
81-95	FHPEDTGQVFQVS	203-217	TGGKSTCGDGGPL
83-97	PEDTGQVFQVS	205-219	GKSTCGDGGPLV
85-99	DTGQVFQVS	207-221	STCGDGGPLVCNG
87-101	GQVFQVS	209-223	CSGDGGPLVCNGV
89-103	VFQVS	211-225	GDSGGPLVCNGVL
91-105	QVSHSFPHPLYDMSL	213-227	SGGPLVCNGVLQGIT
93-107	SHSFPHPLYDMSLLK	215-229	GPLVCNGVLQGITSW
95-109	SFPHPHYDMSLLKNR	217-231	LVCNGVLQGITSWGS
97-111	PHPLYDMSLLKNRFL	219-233	CNGVLQGITSWGSEP
99-113	PLYDMSLLKNRFLRP	221-235	GVLQGITSWGSEPC
101-115	YDMSLLKNRFLRPGD	223-237	LQGITSWGSEPCALP
103-117	MSLLKNRFLRPGDDS	225-239	GITSWGSEPCALPER
105-119	LLKNRFLRPGDDSSH	227-241	TSWGSEPCALPERPS
107-121	KNRFLRPGDDSSHDL	229-243	WGSEPCALPERPSLY
109-123	RFLRPGDDSSHDLML	231-245	SEPCALPERPSLYTK
111-125	LRPGDDSSHDLMLLR	233-247	PCALPERPSLYTKVV
113-127	PGDDSSHDLMLRLRS	235-249	ALPERPSLYTKVVHY
115-129	DDSSHDLMLRLSEP	237-251	PERPSLYTKVVHYRK
117-131	SSHDLMLRLSEPAAE	239-253	RPSLYTKVVHYRKWI
119-133	HDLMLRLSEPAAELT	241-255	SLYTKVVHYRKWIKD
121-135	LMLLRSEPAAELTDA	243-257	YTKVVHYRKWIKDTI
123-137	LLRLSEPAAELTDAVK	245-259	KVVHYRKWIKDTIV
125-139	RLSEPAAELTDAVKVM	247-261	VHYRKWIKDTIVANP
127-141	SEPAELTDAVKVMDL	249-261	YRKWIKDTIVANP
		251-261	KWIKDTIVANP

104

TABLE 26

5	PSCA peptide pool: The amino acid position and sequence of fifteen amino acid peptides overlapping by thirteen amino acids from PSCA peptide library is shown.	
	amino acid no.	PSCA peptide sequence
	1-15	MKAVALLLMAGLAL
10	3-17	AVLLALLMAGLALQP
	5-19	LLALLMAGLALQPGT
	7-21	ALLMAGLALQPGTAL
15	9-23	LMAGLALQPGTALLC
	11-25	AGLALQPGTALLCYS
	13-27	LALQPGTALLCYSCK
20	15-29	LQPGTALLCYSCKAQ
	17-31	PGTALLCYSCKAQVS
	19-33	TALLCYSCKAQVSNE
25	21-35	LLCYSCKAQVSNEDEC
	23-37	CYSCKAQVSNEDEC
	25-39	SCKAQVSNEDEC
	27-41	KAQVSNEDEC
30	29-43	QVSNEDEC
	31-45	SNEDCLQVENCTQLG
	33-47	EDCLQVENCTQLGEQ
35	35-49	CLQVENCTQLGEQCW
	37-51	QVENCTQLGEQCWT
	39-53	ENCTQLGEQCWTARI
40	41-55	CTQLGEQCWTARI
	43-57	QLGEQCWTARI
	45-59	GEQCWTARI
45	47-61	QCWTARI
	49-63	WTARI
	51-65	ARIRAVGLLTVISK
50	53-67	IRAVGLLTVISK
	55-69	AVGLLTVISK
	57-71	GLLTVisKGCSLN
55	59-73	LTVISKGC
	61-75	VisKGCSLN
	63-77	SKGCSLN
60	65-79	GCSLN
	67-81	SLNCVDDSQDYYVG
	69-83	NCVDDSQDYYVGK
	71-85	VDDSQDYYVGKKN
65	73-87	DSQDYYVGKKN

105

TABLE 26-continued

PSCA peptide pool: The amino acid position and sequence of fifteen amino acid peptides overlapping by thirteen amino acids from PSCA peptide library is shown.

amino acid no.	PSCA peptide sequence
75-89	QDYYVGKKNITCCDT
77-91	YYVGKKNITCCDTDL
79-93	VGKKNITCCDTDLCN
81-95	KKNITCCDTDLCNAS
83-97	NITCCDTDLNCNASGA
85-99	TCCDTDLNCNASGAHA
87-101	CDTDLNCNASGAHALQ
89-103	TDLCNASGAHALQPA
91-105	LCNASGAHALQPAAA
93-107	NASGAHALQPAAAIL

106

TABLE 26-continued

PSCA peptide pool: The amino acid position and sequence of fifteen amino acid peptides overlapping by thirteen amino acids from PSCA peptide library is shown.

amino acid no.	PSCA peptide sequence
95-109	SGAHALQPAAAILAL
10	97-111
	AHALQPAAAILALLP
	99-113
	ALQPAAAILALLPAL
	101-115
	QPAAAILALLPALGL
15	103-117
	AAAILALLPALGLLL
	105-119
	AILALLPALGLLLWG
	107-121
	LALLPALGLLLWGPG
20	109-123
	LLPALGLLLWGPQQL
	111-125
	PALGLLLWGPQQL

TABLE 27

IFN γ T cell responses induced by the single antigen (Group 1: Ad-PSMA; Group 2: Ad-PSA; Group 3: Ad-PSCA; Group 4: mix of Ad-PSMA, Ad-PSA and Ad- PSCA) or triple antigen expressing adenovirus vectors (Group 4: Ad-733; Group 6: Ad- 734; Group 7: Ad-735; Group 8: Ad-796 and Group 9: Ad- 809) after adenovirus prime with anti-CTLA-4 analyzed by ELISPOT assay.

Response to PSMA		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	2356	988	1505	335	501	2145	NA	NA
	2	342	1776	154	329	158	438	321	NA
	3	0	1276	40	126	20	0	NA	NA
	4	304	1198	774	2007	1277	1310	1159	2774
	5	943	2670	2757	780	1082	2251	1566	544
	6	472	2092	4248	1369	1760	2964	1447	263
	7	2161	2202	939	869	3513	1654	3424	900
	8	1166	799	2566	663	1043	497	1334	560
	9	1621	3247	2031	980	2942	1882	1918	3805
Response to PSA peptides		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	0	0	0	48	0	42	NA	NA
	2	1419	1426	298	1223	1346	1120	1694	NA
	3	6	462	91	0	77	0	NA	NA
	4	790	1093	1611	790	186	783	2016	1964
	5	101	510	955	665	336	1512	1052	119
	6	236	673	2155	724	504	1600	930	83
	7	0	1086	494	663	2265	117	1712	84
	8	1893	2060	1490	1759	2352	1700	2232	1326
	9	1193	1432	207	1738	1886	949	492	1940
Response to PSCA		animal ID							
		1	2	3	4	5	6	7	8
Group No.	1	795	425	874	1069	219	203	NA	NA
	2	669	713	391	199	164	560	461	NA
	3	510	1234	1099	1115	1194	339	NA	NA
	4	778	528	680	1101	165	531	1175	1009
	5	378	1061	1161	143	71	756	766	204
	6	118	380	1190	403	829	1225	148	261
	7	615	1141	794	564	1175	490	856	204
	8	968	1136	745	290	550	976	955	841
	9	929	434	1150	745	1120	246	1195	970

TABLE 28

IFN γ T cell responses induced by the single antigen (Group 1: PSMA; Group 2: PSA; Group 3: PSCA; Group 4: mix of PSMA, PSA and PSCA) or triple antigen expressing vectors (Groups 5-9) after adenovirus prime with anti-CTLA-4 and DNA electroporation boost immunizations analyzed by ELISPOT assay.

Response to PSMA		animal ID							
	peptides	1	2	3	4	5	6	7	8
Group No.	1	1327	1535	1643	535	1506	1267	NA	NA
	2	15	266	26	191	10	46	1305	NA
	3	0	445	5	75	4	6	NA	NA
	4	365	675	731	1134	244	714	999	1683
	5	270	1623	2254	626	860	2245	1453	1046
	6	541	1151	2923	1094	1061	1746	691	489
	7	1183	1183	1453	1649	2844	1470	2321	991
	8	486	69	399	216	351	758	416	1389
	9	1430	2631	2015	475	1368	1826	1851	3141
Response to PSA		animal ID							
	peptides	1	2	3	4	5	6	7	8
Group No.	1	0	0	0	1	0	26	NA	NA
	2	1883	1236	1574	393	461	941	1565	NA
	3	33	30	9	13	8	11	NA	NA
	4	571	1129	1180	210	88	274	924	360
	5	50	1255	1344	628	210	638	948	1161
	6	88	228	1390	489	1006	908	683	51
	7	0	211	321	156	1509	56	199	85
	8	414	611	85	105	544	1080	331	1883
	9	434	821	556	343	1160	510	144	1115
Response to PSCA		animal ID							
	peptides	1	2	3	4	5	6	7	8
Group No.	1	615	799	533	74	258	61	NA	NA
	2	194	170	133	133	8	66	405	NA
	3	819	1071	873	839	1045	724	NA	NA
	4	543	506	664	470	70	673	761	1235
	5	154	455	1218	109	218	1094	285	569
	6	56	293	603	506	745	911	63	165
	7	429	298	939	589	1226	263	803	451
	8	279	214	871	61	144	511	193	963
	9	379	191	1196	73	699	198	616	836

Example 20

Reduction of STAT3 Phosphorylation by Sunitinib

The following example is provided to illustrate the capability of sunitinib to directly inhibit the phosphorylation of STAT3 (signal transducer of activator of transcription 3), a key mediator of immune suppression in the spleen.

Study Procedure.

The acute effect of Sutent on the phosphorylation status of STAT3 in the spleen was investigated in a subcutaneous tumor mouse model to evaluate the direct immunomodulatory effects of the compound. Briefly, 10-12 week old female BALB/neuT mice were implanted with 1e6 TUBO cells subcutaneously in the right flank. After forty one days post tumor implant, Sutent was given by oral gavage at 20 mg/kg. At 0, 1, 3, 7 and 24 hrs post Sutent dosing, three animals per timepoint were sacrificed under IAUCUC guidelines and spleens were immediately snap frozen in liquid nitrogen to preserve the phosphorylation status. Spleens from female BALB/c mice were snap frozen to use as healthy mice controls.

STAT3 Assay Procedures.

Snap frozen spleens were homogenized at 100 mg tissue per 500 μ L lysis buffer (70 mM NaCl, 50 mM β -glycerol phosphate, 10 mM HEPES, 1% Triton X-100, 100 mM Na₃VO₄, 100 mM PMSF, 1 mg/mL leupeptin) using a

polytron tissue homogenizer. Resulting digests were centrifuged at 10,000 g for 15 minutes. The supernatant was isolated and protein concentrations were determined using

BCA protein assay kit (Pierce, Rockford, Ill.). Forty micrograms of protein were added to each well of either a total STAT3 (eBioscience, cat no. 85-86101-11) or phosphor-STAT3 (eBioscience, cat no. 85-86102-11) ELISA Kit. Relative levels of either protein were compared with standards provided in the kit and with standards purchased independently (Signaling Technologies, cat no. 9333-S).

Results.

Table 29 shows the result of a representative STAT assay that evaluates the effect of Sutent on the phosphorylation status of STAT3 in the spleen. Both spleen extracts from healthy or tumor bearing mice exhibited similar levels of STAT3 protein by ELISA (Total STAT3). However, compared to healthy BALB/c, the extracts from tumor bearing mice had significantly higher levels of phosphorylated STAT3 (Student's T-test, P<0.001). The phosphorylation levels rapidly decreased to levels similar to healthy animals only 1 hr after Sutent treatment and maintained at lower levels than the untreated mice up to 7 hrs. At 24 hrs the phosphorylation levels of STAT3 completely recovered to levels before Sutent treatment. The phosphorylation kinetics mirrors the levels of circulating Sutent in the blood. The rapid response of STAT3 phosphorylation in the spleen

reflecting the pharmacokinetic profile of Sutent suggests a direct immunomodulatory function of Sutent in tumor bearing animals.

TABLE. 29

The relative levels of phosphorylated STAT3 and total STAT3 from healthy BALB/c and tumor bearing BALB/neuT mice before or after Sutent treatment at multiple time points.								
Strain	Time	Phospho-STAT3			Total STAT3			
		Point hrs	Individual values	Mean	SEM	Individual values	Mean	SEM
Balb/c	0	0.11	0.09	0.01		0.12	0.13	0.00
		0.08				0.12		
		0.09				0.14		
	1	0.26	0.23	0.02		0.12	0.09	0.01
Tumor bearing BALB/neuT	0	1.08	1.31	0.12		0.08	0.09	0.01
		1.38				0.11		
		1.46				NA		
	1	0.25				0.08		
		0.19				0.08		

TABLE. 29-continued

The relative levels of phosphorylated STAT3 and total STAT3 from healthy BALB/c and tumor bearing BALB/neuT mice before or after Sutent treatment at multiple time points.							
Strain	Time	Phospho-STAT3				Total STAT3	
		Point	Individual values	Mean	SEM	Individual values	Mean
10	3	hrs	0.19	0.19	0.02	0.15	0.13
			0.15			0.09	
			0.22			0.16	
	7	hrs	0.19	0.27	0.04	0.10	0.08
			0.31			0.07	
			0.29			0.08	
	24	hrs	1.54	1.44	0.07	0.08	0.08
			1.30			0.08	
			1.47			0.08	

RAW SEQUENCE LISTING

SEQ ID NO : 1. AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSMA
 MNWNLHETDSAVATRPRWLCAAGALVLAGGFFLLGFLFGWIKSNEATNITPKHN
 MKAFLDELKAENIKKFLYNTQIPLHAGTEQNPLAKQIQSOWQFGLDSELAYHDV
 LLSYPNKTHPYIISIINEDCNEIINTSLFEPPPPGYENVSIDVPFSAFSPCGMPGECGL
 VVNYARTEDFFKLERDMKINCNSKGIVIARYGKVFRGNKVKNQLAGAKGVILYSDPA
 DYFAPGVKSYPDGWNLPGGVGQRGNILNLNLNGAGDPLTPCGY PANEYAYRGRJAAEVG
 LPSIPVHPIGYYDAQKLLKEMGGSAPPDSSWRSGLSKVPYNNPGPFTGFNSTQVKM
 HIHSNEVTRVLYNVIQTLRGAEVDPYRVLGGHRSWVFGGIDPQSAAVHEIVRSF
 GTLKKEGWWRPRRTILFASWDAAEFGLLGSTWEAENSRLLQERGVAYINADSSIEGN
 YTLRVDCTPLMSVHLNLTKEKLPSDEFGEKSLYESWTKKSPESEFGMPRISKLG
 SGNDPEVFPQRGLIASGRAYTCKNWETNKPSGTYLHSVYETVYELKEVDFPMKYH
 LTVAQVRGGMWFELANSIVLPFDRCRDYAVVLRKYADK1YSISMKHQPQMKTYTSVSDS
 LFSAVKNFTETIAASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFIGDPLGLPDRPFYRH
 VIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAFTVQAAAETLSE
 VA

SEQ ID NO : 3 . AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 1
MASARRPRWLCA GALVLAGGFLLGFLFGWFIKSSEATNISPQHNVKAFLDEMKAE
NIKKFLYFLTQIPHLAGTECNFQLAKQIQA EKWFGLSDVSELAHYDVLVSYNPENTHPNY
ISI IDEDGNEIFNTSLFEPPPPGYCNKUUSVPPVYEFASFPQGMPEGDLVYVNYARTEDFF
KLEPLERKINGSKULLJARYGVKCPNGNKVNUOAGAKGTLSYSPDARPAVGKVSYPDC

- continued

RAW SEQUENCE LISTING

WNLPGGGVQRGNVLNNGAGDPLTPGYPANEYARRELAEAVGLPSIPVHPIGYYDA
QKLLKEKMGGSAPPDSSWKGSLVKPVNVGPFGTGFNSTQVKMHIHSNEVTRIYNVI
GTIRGAVEPDYRVILVGHHRAWWFVGGIDPQSGAAVHVEIFRSVGTLKKKGWRPRRTII
FASWDAAEEFLGGGSTWEAENSRLLQERGVYAINADHSIIEGNYTLRVDCTPLMSLV
YNTLTKELQSPDEGFEKGSLYESWTKKSPSPEFSGVPRINKLGSNDPEVFFQRLGIAS
GRARYTKWNKTNKFSGYPLYHSVYETYELVEKFYDPMFKYHHTVAQVRGGLVFELAD
SIVLPFDQDYAVVLRKYADK1YLNLMKHKPEELKTYSVSFDSLFSAVKNFTETIASKFNO
RLQDFDKCNPPLVRLMNLQMLFERAVFDPLGLPDRPFYRHVIYAPSSHNKYAGESF
PGYIYDALFDIEKVDPKSCKAWGEVKRO1YVAAPTVOAAEELTSEVA

SEQ ID NO: 4 . NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE

OF PSMA SHUFFLED ANTIGEN 1 OF SEQ ID NO: 3

SEQ ID NO: 5. AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 2

MASARRPRWL CAGALVLAGGFLLGFLFGWFIKSSSEATNITPQHNVKAFLDELKAEN
1KKFLYNTQIPHLAGTEQNFELAKQIQAQWKEFGLDSVELSHYDVLLSYPNEPTHPNV
SIIDEDGNEI FNTSLFPEPPPGYENISDVPVPPYSAFSPQGMPEGLDVLYVNYARTEDFFK
LERDMKINCSKGKLLIARYGVFKFRGNVKVNQLAGAKIGLILYSPDADYFAPGVKSYPDG
WNLPGGGVQRGNVLNNGADPLTPGYPANEYARRGIAEAVGLPSIPVHPIGYYDA
QKLLEKMGAAAPPDSSWKGSQLPVNVPGFTGNFSTQVKMHIHSTNEVTRIYNVI
GTLKGAPEPDVRYL1GGHRDAWVFGGIDPQSGAAVHVIEIURSFGTLLKKGWRPRRTI
LFASWDAAEEFGLLGSTEWAENSRLLQERGVAYINADSSIIEGNYTTLRVDCTPLMYSL
VYMLTKEQLSPDEGEFEGKSLFDWSTEKSPSPFESGLPRISKLGSGNDFEVFFORLGI
SGRARYTKDWTKTSKFSGYPLHVSYETYELVEKFYDPMFKYHLTVAQVRGGIVFELA
NSVVLPLFCQDYAVVLLKVKYADK1YNI SMKHPQEMKTYSVSFDSLFSAVKNFTEIASKF
NQLRQDFDKNNP L1LRRMMNDQLMFLERAFDPLGLPDRPFYRHVYIAPSSHNKYAGES
FPGIYDALF1EISVKDPSKAWGEVKRQ1YVAATVTOOAAETLSEVA

SEQ ID NO: 6. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE

SEQ ID NO: 6. NUCLEOTIDE SEQUENCE ENCODING PSMA SHUFFLED ANTIGEN 2 OF SEQ ID NO:

atggctagccgcaggacggcccgatggctgtgtggccctgggtgtggctggccgttttcctgtggcttcgtt
cggtgttcatcaagagcagcagcggccacaacatccccccagcacaacgtgaaggcttctggacag
ctgaaggccgaaatatacaaaggacttccgttacaacttccacccatccccccatggccggcaccgacgaaactc
gagctggccaaagcagatccaggcccagtggaaagagttcggcttgacagcgtggaaactggccactacgacgtgc
tgtgtgtactcccaacggacacaccccaactatcatcagatcatcgcacggacggccaaacggagatttcaaccc
agctgttgcgccccctccacccgcgtacgagaacatcagcgcacgtgtgtccccctacagccattcagtcacag
ggaatgcccggggcgcacctgtgtacgtgaactacgccccggaccggaggacttctcaagtgaaacggacatgaa
gatcaactgcagcggcaagatctgtatcggccagatcggcaagggtttccggggcaacaaagtgaagaacggcc
ctggcaggccggccaaacgttcatcgtgtacgcggccgcgtacttccggcttgcgtgtacccatccccgg
ggctggaaactcgctcgccggaggctcagggggcaacgtgtgtacccatcgccgtgtccatccccgg
ctggatcccccccaacggatcgcctacacagcggggaaatcgccggaggccgtggccctgtcgtacatccctgtcac
ccatcggtactacgcgcggaaactgtggaaaagatggggggagccggccctccggacagcttggaaagg
gcagcgtgcagggtccctacaacgtggccctgggttccacggcaacttacgcacccagaagaatgtgaagatgcac
acacgcaccaacgaagtgcacccggatctacaacgtgtatcggtacccctgaaggccgcgtggaaacccgcacagatacg
gtatcgccggccggccacgggcgtgggttgcggaggatcgcacccatcagggccgcgtgcgtgtgcac
tgcgtggggaggacttgcgtactacagaagaagggttggccggccacggaccatctgttgcgcacttggacgc
ggagaatttccggcttgcggccggccacggcggaggaaacatgtggccgtgtgcggaggaaacccatcgcc
acatcaacgcggacacgcacatcgaggcaactacaccctcgccgggtggactgcacccctgtatgtacacgcctgtg

-continued

RAW SEQUENCE LISTING

tacaacctgaccaaagagctgcagagccccacgcaggggctcgagggcaagtccctgttgcactctggaccgagaa
 gtcggcccgcccgaggctcagggcctgcccagaatcagaacgcggcggcacaactcgagggtttcttcca
 gggctggaaatcgccagccggaggcccgatcaccaaggactggaaaacccagcaacttcccgcttacccctg
 taccacagcgttacgagacatcagactggggaaatgttctacgaccggccatcgtaacttgcacccatgc
 aggtccgaggccgcattgttcaactggccacagcgttgcattcgatgtcaggactacgcgtggtgcc
 aagaagtacgcgcacaaatctacaacatcagcatgaagcaccccccaggaaatgaaaactacagcgttgc
 acagcgttccgcggcgttcaaggaaatcaccggatgccttcaaccaggactgcaggacttcgacaaga
 acaaccccatctgtccggatgtatgttctggaaacggccatcgaccccttgcctgccc
 cgaccggcccttttacccgcactgtatctatggcccaacaaatacgcggcggaggatcccccc
 ctacgatgcccttgcatacgagacaagggtggacccaggacggcctggggagaatgaaagcggc
 ggcgcattcacagtgcaggctgtccgcagacactgagcggagggtggcc

SEQ ID NO: 7. AMINO ACID SEQUENCE OF PSMA SHUFFLED ANTIGEN 3
 MASARRPRWLCAAGALVLAGGFFLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAE
 NIKKFLYNTFQIPLLAGTEQNQFQLAKQIQSQWKEFGLDSVELAHYDVLLSYPNKTHPN
 YISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLVNVYARTEDF
 FKLERDMKINCNSKIVIARYGVFRGNVKVNAOLAGAKGVILYSDPADYFAPGVKSYP
 DGWNLPGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPPIGY
 DAQKLLEKMGGSAPPDSWRGSLKVPYNVPFGFTGNFSTQKVMHIHSTNEVTRIY
 VIGTLRGAVEPDYRVLGGHRSWVFGGIDPQSGAAAVVHEIVRSFGTLLKEGWPR
 TILFASWDAEEFGLLGSTWEAENSRLLQERGVAYINADSSIIEGNYTLRVDCTPLH
 LVYNLTKEKSPDEGEGKSLYESWTKKSPPEFSGMPRISKLGSNDFEVFFQRLGI
 SSGRARYTKDWKTSKFSSYPLHISIETYELVVKFYDPMFKYHLTVQVRGMVTEL
 ANSIVLPFDRCRDYAVALKNHAENLYSISMKHPOEMKTYSVSFDSLFSAVKNFTEIA
 SERLQDFDKSNPPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGES
 FPGIYDALFDIESKVDPSKAWGEVKRQIYVAATVQAAAETLSEVA

SEQ ID NO: 8. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE
 OF PSMA SHUFFLED ANTIGEN 3 OF SEQ ID NO: 7
 atggctagcgcacagcggccagatggctgtgtctggccctggctggccgttttctgttgcggccctgtt
 cggctgttcatcaagacgcacgcggccaccaatcaccccaagcacaatcatgaaggcccttctggac
 ctggaggccgagaaatcaagaatctgttcaacttccacatggccatccggccacttggccggc
 cggccatccggccacatggccatccggccacttccacatggccatccggccacttggcc
 tggccatccggccacatggccatccggccacttggccatccggccacttggccatccggcc
 agcgttgcggccatccggccacttggccatccggccacttggccatccggccacttggcc
 gaatggccggggcacttggccatccggccacttggccatccggccacttggccatccggcc
 atcaactgcggccatccggccacttggccatccggccacttggccatccggccacttggcc
 tggccggccatccggccacttggccatccggccacttggccatccggccacttggcc
 gcttgcggccatccggccacttggccatccggccacttggccatccggccacttggcc
 ggctatccggccatccggccacttggccatccggccacttggccatccggccacttggcc
 catcggtactacgcggccatccggccacttggccatccggccacttggccatccggcc
 gcttgcggccatccggccacttggccatccggccacttggccatccggccacttggcc
 gaaatggccatccggccacttggccatccggccacttggccatccggccacttggcc
 gcaacaccaaaatggccatccggccacttggccatccggccacttggccatccggcc
 gcttgcggccatccggccacttggccatccggccacttggccatccggccacttggcc
 ctggccatccggccatccggccacttggccatccggccacttggccatccggccacttggcc
 gatcgccatccggccatccggccacttggccatccggccacttggccatccggcc
 gatcgccatccggccacttggccatccggccacttggccatccggccacttggcc
 tcaacacccatcgccatccggccacttggccatccggccacttggccatccggcc
 aacctggccatccggccacttggccatccggccacttggccatccggccacttggcc
 tcccccacccggccatccggccacttggccatccggccacttggccatccggcc
 cgccatccggccatccggccacttggccatccggccacttggccatccggcc
 accacacccatcgccatccggccacttggccatccggccacttggccatccggcc
 ggtccggccatccggccacttggccatccggccacttggccatccggccacttggcc
 aagaaccacccatcgccatccggccacttggccatccggccacttggccatccggcc
 acacccatcgccatccggccacttggccatccggccacttggccatccggcc
 gaaacccatcgccatccggccacttggccatccggccacttggccatccggcc
 cgaccggccatccggccacttggccatccggccacttggccatccggcc
 ctacgatccggccatccggccacttggccatccggccacttggccatccggcc
 tggccgcattcacagtgcaggccgttgcggccacttggccatccggcc

SEQ ID NO: 9. AMINO ACID SEQUENCE OF A MEMBRANE-BOUND PSMA
 ANTIGEN
 MASARRPRWLCAAGALVLAGGFFLLGFLFGWFIKSSNEATNITPKHNMKAFLDELKAE
 NIKKFLYNTFQIPLLAGTEQNQFQLAKQIQSQWKEFGLDSVELAHYDVLLSYPNKTHPN
 YISIINEDGNEIFNTSLFEPPPPGYENVSDIVPPFSAFSPQGMPEGDLVNVYARTEDF
 FKLERDMKINCNSKIVIARYGVFRGNVKVNAOLAGAKGVILYSDPADYFAPGVKSYP
 DGWNLPGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPPIGY
 DAQKLLEKMGGSAPPDSWRGSLKVPYNVPFGFTGNFSTQKVMHIHSTNEVTRIY
 NVIGTLRGAVEPDYRVLGGHRSWVFGGIDPQSGAAAVVHEIVRSFGTLLKEGWPR
 RTILFASWDAEEFGLLGSTWEAENSRLLQERGVAYINADSSIIEGNYTLRVDCTPLM
 SLVHNLTKEKSPDEGEGKSLYESWTKKSPPEFSGMPRISKLGSNDFEVFFQRLGI
 GIASGRARYTKNWETNKFSGYPLHISVYETYELVKEFKYDPMFKYHLTVQVRGMV
 FELANSIVLPFDRCRDYAVVLRKYADKIYSISMKHPOEMKTYSVSFDSLFSAVKNFTEIA
 SKFSERLQDFDKSNPPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKY
 GESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAATVQAAAETLSEVA

-continued

RAW SEQUENCE LISTING

SEQ ID NO: 11. AMINO ACID SEQUENCE OF A CYTOSOLIC PSMA ANTIGEN
MASKSSNEATNITPKHNMAFLDELKAENIKKFLYNTFTQIPLLAGTEQNQFLAKQIQSQ
WKEFGFLDSVELAHYDVLLSPYTPNQYIISIINEDGNEIFNTTSLFEPPPPQGYENVSIDVP
PFSAFSPQGMPGEGDVLVNVYNTKEDFFKKLERDMKINCSGKIVIARYQGVFRGNKVKN
AQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQRGNILNLNLNGADGPLTPGY
PANEYAYRRGIAEAAGLPSIPVHPIGYYDAQKLLEKMGGSAPPDSSWRGLSKVPPNV
GPFGPTGNGSTQVKVMIHNTSTNEVTIRYNTLRLGAAVEPDYVILGGHRDWSVFGGID
PQSGAAVVHEIVRSFTGLKEGWRPRRTLIFASWDAEEFGLLGSTEWAENSRLLQE
RGVAYINADSSIEGNYTILRVDTCLPMYSLVHNLTKEKLSPDEGFECKSLYEWTKHPSP
SPEFSGMPRIASKLGSGNDFEVFVQRLGIASGRARYTKNWETNKFSGYPLYHSVYETY
ELVEKFYDPMFYKHLTVAQVRGGMVFELANSIVLPFDCCRDYAVVLRKYADKIYSISMK
HQPEMKTYTSVFSDFLSFPAVKNFTIEIASKFSERLQLQDFDKSNPIVLRMMNDQLMFLERA
IDPLGLPDRPFYRHVIYAPSSHNNKYAGESFPGIIYDALFDIESKVDPSKAWGEVKRQIYV
AAFTVQAAAETLSEVA

-continued

RAW SEQUENCE LISTING

SEQ ID NO: 13. AMINO ACID SEQUENCE OF A SECRETED PSMA ANTIGEN
 MASETDTLLWVLLWPGSTGDAAKSSNEATNI TPKHNMKAFLDELKAENIKKFLYN
 FTOIPHLAGTEQNFQLAKIQSOWKEFGLDSVELAHYDVLSSYPNKTHPNYIINNEDG
 NEIFNTSLFEPPEPPGYEVNSDVPFFPSAFS PQGMPEGDLYVYVNARTEDFFKLERDMK
 INCSGKVIIARYGVFKRGNVKQNAQAGKGVILYSDPADYFAPGKVSKYSPDGWNLPG
 GGVQRGNI LNLngAGDPPLTPGVPANEYARRGIAEAVGLPSIPVHPIGYYDAQKLLEK
 MGGSAPPDSWRGSLSKVVPYNVPGPCTGNGSTQKVKMHIHSTNEVTRIYVNIGTLRGA
 VEPDRYVILGGHRDSWVGFGIDPQSGAAVHVIEURSFGLTKKEGWRPRTRILFASWD
 AEEFGLLGSTEWABEANSRLLQERGVAYINADSSIEGNYTLRVDCTPLMSYLVHNLTKE
 LKSPDGEFGKSLYESWTKSKSPFEGMSPRI SKLGSGNDFEVFFQRLGIASGRARY
 TKNWETNKFSGYPLYHSVYETYELVEKFYDPFMKYHLTVAQVRGGMVFELANSIVLP
 FDCDRYAVLRLRYADK1YSISMKHPQEMKTYSVSFDLSLEAVKNTFEIASFKSERLQD
 FDKSNP1VLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYD
 ALEDIEKVDPSKAWGKVROJYVAFTVOAAAETLSEVA

SEQ ID NO: 14. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF THE SECRETED PSMA ANTIGEN OF SEQ ID NO: 13

SEQ ID NO: 15. AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSA
MASWVPVFVFTLSVTWIGAAPLILSRLIVGGWECEKHSQPWQLVASRGRAVCGGVLV
HPQWVLTAACIRCNKSVLLGRRSHFPHPDGTQVQVQVSNSFPHPFLYDMSLLKNRFLRP
GDDDSHHDLMLRLRSPEAELTDAVKVMDLTQEPALGTTCYASGWGSIEPEEFLTPKK
LQCVLDLHV1SNDVCAQVHPQKVTKMCLC4RGRTWGGKSTCSDGSGPLVCNGVLQGI
TSWGSEPCALPERPSLYTVHRYRKWIKDTIVANP

SEQ ID NO: 16. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSA OF SEQ ID NO: 15

SEQ ID NO: 17. AMINO ACID SEQUENCE OF A CYTOSOLIC PSA ANTIGEN
MASIVGGWECEKHSQPVOLVVASRGRAVCGVLVHPOWLTAACIRNKSVILLGR

HSLFHFEDTGQVFQVSHSFPFLYDMSLLKNRFLRPGDDSSHDLMLRLSEPAELTD
AVKVMGLPQTQEPAFLTTCYASGWSIEPEEFLTPKKLQCVDLHV1SNDVCAQVHPQK
VTFKMCLCAGRWGKGSTCGSDGGPLVCNGVLQGITSWGSPECALPERPSLYTKVV
HYRKW1KDTIVANP

SEQ ID NO: 18. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE

OF THE CYTOSOLIC PSA ANTIGEN OF SEQ ID N

atggctagcattgtggaggcgccggagtgcgagaagcattcccaaccctggcagggtgcttgtggctctgtggcagggg
cagtcgtcgccgcgggttcttgtcacccccacaggtccatcacagctgcactcatcaggaaacaacagctgtatcttgc
ctgggtcgccacagcttgttcatcctgaagacacagggcaggatttcaggtaaggctaggccacagcttccccacccgccttac

-continued

RAW SEQUENCE LISTING

SEQ ID NO: 19. AMINO ACID SEQUENCE OF A MEMBRANE-BOUND PSA ANTIGEN
MASARRPRWLCAAGALVLAGGFLLGLFLFGWFIKSNEATNTIPGIVGGWECEKHSQP
WQVLVASRGRARAVCGGVLVHPQWLTAACIRNKSVILLGRHSLFHPEDTGQVFQVS
HSFPHPLYDMSLLKNRFLRPGDDSSHDLMLRLSEPAELTDAVKVMDLPTQEPAALGT
TCYASGWNSIIEPEEFLTPKQLCQVDLHV1SNDVCAQVHNPQKVTKFMLCACRGWTKGK
STGCSGSCPLVNCNGQCLITTSWCEC71PERPFLVYVWVWVWVWVWVWVWVWVWV

SEQ ID NO: 21. AMINO ACID SEQUENCE OF THE FULL LENGTH HUMAN PSCA
MASKAVLLALLMAGLALQP GTT ALLCYS CKAQV SNED CLQ VEN TQL GEC QWTARIR A
VG LLTVISKGCSLNCV DSD QD YVVG KKN ITCC DTD LCN ASGA HAL QPAA ILL P AL
G J L L W Q P G C O T

SEQ ID NO: 22. NUCLEOTIDE SEQUENCE ENCODING AMINO ACID SEQUENCE
OF THE FULL LENGTH HUMAN PSCA OF SEQ ID NO: 21
atggctagaacaggctgtcgtgcgtccctgtatggcaggcttgcgcctgcagccaggcactgcgcctgtgtactcc
gaaaggcccaagggtgacaaacggaggactgcgtcccgagggtggaaactgcaccccaggctgggggagcagtgtgcggaccg
cgcgcatccgcgcagttggcctctgaccgtcatcagcaaaggctgcagcttgactgcgtggatgactcacaggacta
ctacgtggggaaaaggactacgtgttcacccgtttgtcaaccgcacccgcggggccatgcctgcaggg
ctaccccccatttcggccatcgttcacccgtttgtcaaccgcacccgcggggccatgcctgcaggg

SEQ ID NO: 23. NUCLEOTIDE SEQUENCE OF PLASMID 5166
GGCGTAATGCTCTGCCAGTGTACAAACCAATTAAACCAATTCTGATTAGAAAAACTC
ATCGAGCATCAAATGAACCTGCAATTATTCATATCAGGATTATCAACCATTT
TTGAAAAGCCGTTCTGTAAAGGAGAAAACCTACCGAGGCGATTCCATAGG
ATGCGAACATCTGGTATCGCTCTGGATTCTGCATCCTGGCAACATCAAC
CTATTAACTTCCCTCGTCAAAAATAAGGTATCAAGTGAGAAATCACCATGAGTG
ACGACTGAATCCGGTGGAAATGCCAAGGGTTATCGATTCTTCCAGACTTGTTC
ACGAGGCCGACCCATTACGCTCGTCATCAAATCACTCGCATCAACAAACCGTTA
TTCACTTGTGCTGGCCTGGCGACGAAAGCAGAAATACCGCATGCGTGTAAAAGGAC
AATTACAAACAGGAATCAATGCAACGGCGCAGGAACACTGCGAGCGCATCAAC
AATATTTCACTGAATCAGGATATTCTCTAATACCTGGAATGCTGTTTCCCGG
GGATCGCAGTGGTGGATAACCATGCATCATCAGGAGTACCGATAAAATGCTGAT
GGTCGGAAGGGCATAAATTCCGTCAGCGCAGTGTGCTGACCACATCATCTGTA
ACATCATGGCAACGTCACCTTGTGCGATTTGCTTCAAACAAACTCTGGCGCATCGG
GCTTCCCATAACAATCGTAGATTGTCGACCTGATTGCCCCGACATTATCGCGAGC
CCATTATACCCATAAAATCAGGATCCATGTTGGAATTATACCGGGCTCGAGC
AAGAGCTTTCGGTGAATTAGGCTCATACACCCCTTGATTACTGTTATGTAA
CGACAGCGGTGACAATAATTGGCTATTGGCCATTGCGATACGGTGTATCTATCAT
AATATGTACATTATATTGGCTCATGTCACATGACCGGCATGTTGACATTGATTG
TTGACTAGTTAAATAGTAATCAATTACGGGTATTAGTCATAGCCATATATG
GAGTTCCGGTTACATAACTTACGTTAAATGGCCCGCTGGCTGACCGGCCAAC
GACCCCCCGCATTGACGTCATAATGACCTATGTTTCCCATAGTAACGCCAATAG
GGACTTCTTCCATTGACGTCATGGGTGGAGATTATACGGTAAACTCCGCACTTGGC
AGTACATCAAGTGTATCATGCAAGTCGCCCCCTATTGACGTCATGACGGT
AAATGGCCCGCTGGCATTAGGCCAGTACATGACCTTACGGGACTTCCACTT
GCCAGTACATCTACGTTAGTCATCGCTATTACCATGGTGTGCGGTTTGGCA
GTACACCAATGGCCGGTGAAGCGGTTGACTCACGGGATTTCACGGTCTCCAC
CCATTGACGTCATGGGAGTTTGTGTCAGGCAAAATACGGGACTTCCAA
AATGTCGTAATAACCCCGCCCGTGTGACGCAATGGCGTAGGGTGTACGGT
GGGAGGTCTATAAGCAGACTGTTAGTGAACCGTCAGATCGCTGGAGAC
GCCATCCACCGCTTGTGACCTTCATAGAGAACACCGGGACCGATCCAGCTCC
CGCGCCGGGAACGGTCACTTGGAAACCGGGATTCCCGTGGCAAGGTGACTCA
CCGTGGGATCTCAGCAAGCAGGTGTACTCTCCAGGGTGGCCCTGGCTTCCC
CAGTCAAGACTCAGGAGGATTCTGGGGAGCTGTGGGCTCTCTTCAATGTTAC

- continued

RAW SEQUENCE LISTING

TTTGCTTGCTCAACCTGACTATCTCCAGGTAGGATCCCAGAGTCAGGGGT
 CTGTATTTCCTGCTGGTGGCTCAGTCAGAACAGTAAACCCCTGCTCCGAATA
 TTGCCCTCACATCTCGTCATCTCCGAGGACTGGGACCCCTGTGACAAACAT
 GGCTAGCGCGCCGCGCTGGCTGGCTGGCGCTGGCGCTGGGTGCTGGCG
 GGTGGCTCTTCCTCTCGCTCCCTTCGGTGGTTATAAAATCCTCAAATGA
 AGCTACTAACATTACTCCAAAGCATATAATGAAAGCATTTGGATGAATTGAAAG
 CTGAGAACATCAAGAAGTTATAATTACAGATACCACATTAGCAGGA
 ACAGAACAAAACCTTCAGCTTGCAGAACATTCAATCCAGTGGAAAGAATTGG
 CCTGGATTCTGTGAGCTGGCACATTATGATGTCTGTGCTCACCCAAATAAGA
 CTCATCCCAACTACATCTCAATAATTAATGAAAGATGGAATGAGATTTCACACAT
 CATTATTGAATTCCTCCAGGATATGAAAGATGGAATTCAGGATATTGTAACACCT
 TTCACTGCTTCTCTCAAGGAATGCCAGGGCAGTCAGTGTATGTTAACTA
 TGCACGAACACTGAAGACTTCTAAATTGAAACGGGACATGAAAATCAATTGCTCTG
 GGAAAATTGTAATTGCCAGATGAAAAGTTTCAGAGGAATAAGGTTAAAAT
 GCCCAGCTGGCAGGGGCAAAGGAGTCATTCTACTCCGACCCCTGCTGACTAC
 TTGCTCTGGGTGAAGTCTTCCAGATGGTGAATCTTCCGGAGGTGGT
 TCCAGCCTGAAATATCTCAATCTGAATGTTGCAAGGACCCCTCACACAGG
 TTACCCAGCAATGAATATGCTTATAGGCCTGGAATTGCAAGGGCTGTTGGCTT
 CCAAGTATTCTGTTCATCCAATTGGATACTATGATGCACAGAACGCTCCTAGAAAAA
 AATGGGTGCTCAGCACCCAGATAGCAGCTGGAGAGGAAGTCTCAAAGTGCC
 CTACAATGTTGGACCTGGCTTACTGGAAATTCTACACAAAAGTCAGATGC
 ACATCCACTTACCAATGAAGTACAAGAATTACAATGTGATAGTACTCTCAGA
 GGAGCACTGGAACCCAGACAGATATGTCATTCTGGAGGTCACCGGAACTCATGG
 GTGTTGGTGGATTGACCTCAGACTGGAGCAGTCAGTGTGTTCATGAAAATTGTA
 GGAGCTTGGAACACTGAAAAGGAAGGGTGGAGACCTAGAAGAAACAATTGTT
 TGCAAGCTGGATGCAAGAAATTGGTCTCTTGGTCTACTGAGTGGCAGAG
 GAGAATTCAAGACTCTTCAAGAGCGTGGCTGGCTTATAATTAAATGCTGACTCAT
 CTATAGAAGGAAACTACACTCTGAGGTTGATGTCACCCGCTGATGTCACGCTT
 GTGACACAACCTAACAAAGACCTGAAAGCCTGATGAAGGCTTGTGAAAGCAAA
 TCTCTTATGAAAGTGGACTAAAAAAAGTCCTCCCCAGAGTTGAGTGGCATGCC
 CAGGATAAGCAAATTGGATCTGGAAATGATTGAGGTGTTCTCCAACGACTTG
 GAATTGCTTCAAGCAGACAGCAGTAACTCTGAGGAAACATATGAGTTGGAAAAGTTT
 ATGATCCAATGTTAAATACACTCACTGTCGGCCAGGTTGAGGAGATTGCTGAG
 GTTGAGCTGGCCAATTCCATAGTCCTCCCTTTGATTGTCGAGATTATGCTGAG
 TTTAAGAAAGTATGCTGACAAATCTACAGTATTCTATGAAACATCCACAGGAA
 TGAAAGACATACGTGATCAATTGACTTCTGAGTAAAGAATTTCACAG
 AAATTGCTTCAAGTCACTGAGGACTCCAGGACTTTGACAAAAGCAACCCAACT
 GTATTAAAGAATGATGATCACTCATGTTCTGGAAAGAGCATTTATGATCC
 ATTAGGGTTACAGACAGGCCCTTTTATAGGCATGTCATCTGTCAGCAGC
 CACAACAAGTATGCAAGGGAGTCATTCCAGGAAATTATGATGCTGTTGATAT
 TGAAAGCAAAGTGGACCTTCAGGGCTGGGAGAAGTGAAGAGACAGATTAT
 GTGCGAGCTTCAAGCTGGCAGCTGGCAGCTGGAGACTTTGAGTGAAGTAGCTAAA
 GATCTGGGCCCTAACAAAAGATGGGGTATTCCCTAACTTCATGGGTTA
 CGTAATTGAAAGTTGGGGCATTGCCAACAGATCATATTGACAAAAGATCAA
 CACTGTTTAGAAAATTCTGTAAACAGGCCATTGATTGAGGAAAGTATGTCAAAG
 GATTGGTGGTTTGGCTCTGCTCTCCATTACACAAATGTGGATATCTGCC
 TAATGCCCTTGTATGATGTAACAGCTAACAGCTTACGGCTTCACTTCTGCCAACT
 TACAAGGCCCTTCAAGTAAACAGTCATGAAACCTTACCCGTTGCTCGCAAC
 GGCTGGCTGTGCAAGTGTGCTGACGCAACCCCCACTGGCTGGCTGG
 CCATAGGCCATAGCGCATGCGTGGAAACCTTGTGGCTCCCTGCGATCCAC
 TCGGAACCTCTAGCGCTTGTGCTCGCAGGCCGCTGGAGCAAAGCTCATA
 GGAACGTGACAACTCTGCGTCTCGCGGAAATATACATCGTTGAGTCACT
 TGATCTTCTGGCTAAAGGAAATTATTCATTGCAATAGTGTGTTGAAATT
 TGTGTCCTCACTCGGAAGGAATTCTGCATTAAATGAAATCGGCCAACCGCGGGGA
 GAGGCGTTTGGTATTGGGCCCTTCCGCTTCCGCTACTGACTCGCTGC
 GCTCGCTGCTGGCTGGCGAGCGGTATCAGCTCAGTCAGGCTAAAGCGGTAATAC
 GGTATCACAGAAATCAGGGGATAACGAGGAAAGAACATGAGCAGAACAGGC
 AGCAAAAGGCCAGGAACCGTAAAAGGCCGGTTGCTGGCTTTCCATAGGC
 TCGCCCCCTGACGAGCATCACAAAATGACGCTCAAGTCAGAGGTGGCGAA
 ACCCGACAGGACTATAAGATACCAAGCGTTCCCCCTGGAAAGCTCCCTGTC
 GCTCTCTGCTGGCAGCCCTGCGCTTACCGGATACTGTCGCCCTTCTGCC
 GGGAGCGTGGCCTTCTCATAGCTACGCTGAGTGTAGGTTATCTCAGTCGGGTAG
 GTCGTTGCTCCAGCTGGCTGTGTCACGCAACCCCCCTGCGCCGACCG
 TGCGCCTTATCCGTAATCTGCTTGTGAGTCACCCGGTAAGACAGACTTAT
 CGCCACTGGCAGCAGCCACTGTAACAGGATTAGCAGAGCGAGGTATGAGCG
 GTGCTACAGACTCTGAAGTGGTGGCTAACACTACGGCTACACTAGAAGAACAGT
 ATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAGAGTTGGTAGC
 TCTTGTATCGCCAAACAAACCCAGCTGGTAGCGGTTTTTGTGTTGCAAGC
 AGCAGATTACGCGCAGAAAAAGGATCTCAAGAAGATCCTTGTATTTCTACG
 GGGTCTGACGCTCAGTGGAAAGCAAACCTACGTTAAGGGATTGGTCAAG
 TATCAAAAGGATCTCACCTAGATCTTTAAATTAAAGGAAAGTTAAATCAA
 TCTAAAGTATATGAGTAAACCTGGCTGACAGTACCAATGCTTAATCAGTGA
 GCACCTATCTCAGCGATCTGCTATTGCTCATAGTGGCTGACT

- continued

RAW SEQUENCE LISTING

SEQ ID NO: 24. NUCLEOTIDE SEQUENCE OF PLASMID 5259
 GCGCTATGCTTGCCAGTGTACAACCAATTAAACCAATTCTGATTAGAAAACCTC
 ATCGAGCATCAAATGAAACTGCATTATTATTCATACGATTATCAATACCCATATT
 TTGAAAAAGCCGTTCTGTAATGAAAGGAAAACCTACCGAGGCAGTTCCATAGG
 ATGGCAAGATCTGGTATCGCTCTGGACTCGTCAAATCAACAAAC
 CTATAATTCCCTCGTCAAAAATAAGGTATCAAGTGAGAAATCACCATGAGTG
 AGCACTGAGCTGGTGAAGAATGCCAAAAGCTTACGATTTCTTCAGACTTGTTC
 AACAGGGCAGCATTACGCTCGTCAAAATCACTCGCATCAACCAACCGTTA
 TTCATTGTTGATTCGGCTGAGCGAGCAGAACACTCGGATCGTAAAGGAC
 ATTACAAACAGGAATCAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAAC
 ATATTTACCTGTAATCAGGATATTCTTAACTGGAATGCTGTTTCCC
 GGATCCGAGTGGTGAATACCATCGCATCACGGACTACGGATAAAATGTTGAT
 GGTGGAAGAGGCATAAATTCGTCAGCCAGTTAGCTGACCATCTCATCGTA
 ACATCATTGGCAACGCTACCTTGCCATGTTTCAAGAACACTCTGGCGATCGG
 GCTTCCCATATAATCAGTGGCAGCTGTTGGAATTAAATCGGGCTCGAGC
 CCATTATACCCATATAATCAGTGGCAGCTGTTGGAATTAAATCGGGCTCGAGC
 AAGACGTTTCCCCTGTAATAATGGCTCATAAACCCCCCTGTTGATACGTTGAA
 GCAGACAGGTGACAATATTGGCTATTGGCATTCGATACGTTGATCTATATCAT
 ATATGTACATTATATTGGCTCATGTCATGTCATGACCGCCATGTTGACATTGATTA
 TTGACTAGTTAAATAGTAATCAATTACGGGCTTACGGGATTTGACCCATATATG
 GAGTCCGCTTACATAACTACGGTAAATGGCCCGCCTGGCTGACCGCCAAAC
 GACCCCCGCCATTGACGTCAATAATGACGTATGTTCCATAGTAACGCCAATAG
 GGACTTCCATTGACGCTAATGGTGGAGTATTACGGTAAACTGCCACTTGGC
 AGTACATCAAGTGTATCATGCCAGTACATGACCTTACGGACTTCTACTT
 GGCAGTACATCTACGTTAGTCTGCTTACCATGGTATGCGGTTTGGCA
 GTACACCAATGGCGTGGATAGCGGTTTGTACTCACGGGATTCCAAGTCTCCAC
 CCCATTGACGCTAATGGGAGTTTGGCACCATAACGGGACTTCCCAA
 ATATGCTTAATAACCCCGCCCGTTGACGCAAATGGCGTAGGCTGTACGGT
 GGGAGGTCTATAAAGCAGACTCGTTAGTGAACCGTCAATGCCCTGGAGAC
 GCCATCACCGCTGTTTGACCTTACAGAACGACACGGGACCGATCCAGCTCC
 GCGCCGGGAAACGGGATTCCCGTGCAGAAGGTGACTCA
 CGTCCGAGATCTCAGAACGAGGTAGTACTCTCAGGGTGGCTGGCTTCCC
 CAGTCAAGACTCAGGGATTGAGGGACGCTGTTGCTCTTCTACATGACC
 TTTGCTTGCCTCAACCTGACTATCTCCAGGTAGGATCCAGAGTCAGGGT
 CTGTATTTCTCTGGCTTGGCTTACGGTAAACAGTAAACCCCTGCTCCGAATA
 TTGCTCTCATCTGTCATCTCCGGAGGACTGGGACCTGTGACAAACAT
 GGCTAGAAGGCTGTGCTGTTGAGGCTGGCTTGAGCAGGCTGGCTTGAGCC
 AGGCACTGCCCTGCTGTGCAACTGCCCTGCTGAGCCGGCTGGCTTCCC
 CCTGAGGTGGGAAACTGCCAGCTGGGAGCAGCTGGCTGGCTGGCA
 TCCGGCAGTTGGCTCTGACGGTCACTCAGCAAAGGCTGAGCTTGAACGCG
 TGGATGACTCACAGGACTACTACGTGGGCAAGAAGAACATCACGTGCTGTGACAC
 CGACTTGTGCAACGCCAGCGGGCCATGCCCTGAGCCGGCTGCCGCGCATCC
 TTGCGCTCTGCTGCACTGCCCTGCTGTGCTGGGAGCCGGCAGCTATA
 GATCTGGGCTTACAAAAAAAGGGGATTCCCTAAACTCATGGGTTA
 CGTAATTGGAAAGTTGGGGACATTGCCACAAAGTATGTCACAAAGATCAA
 CACTGTTTAAAGGAAACTCTGTAAACAGGCCATTGATTGAAAGTATGTCACAG
 GATTGTTGGCTTTGGCTTGGCTCTCCATTACACAAATGTGGATATCTGGCT
 TAATGCTTTGTATGCTTAACAGCTAACAGCTTACGGCTTCACTTCTGCCAACT
 TACAAGGCCCTTCAAGTAAACAGTACATGAACCTTACCCGTTGCTCGCAAC
 GGCCCTGGCTGTGCAAGGTGTTGCTGACGCAACCCCCACTGGCTGGCTGG
 CCATAGGCCATAGCGCATGCGTGGAAACCTTGTGGCTCCCTGCGATCCATAC
 TCGGAACCTCTAGCGCTTGTGCTCGCAGGCCGCTGGAGCAAAGCTCATA
 GGAACACTGACAACTCTCGCTCTCGGAAATAATACATCGTTGATCTACGTA
 TGATCTTCTTCCCTGCAAAATTATGGGACATCATGAAGGCCCTTGAGCAGC
 TGACTCTGGCTTAAGGAAATTATTTCAATTGCAATAGTGTGTTGAAATT
 TGTGTCCTCACTCGGAAGGAATTGCGATTAATGAATCGGCCAACGCGGGGA
 GAGGCGGTTGGTATTGGGCTCTCCGCTTCCCTGCTCACTGACTCGCTGC
 GCTCGCTGCTGGCTGGGGAGCGGTACAGCTCACTCAAAGGCGGTAAATAC
 GGTATACACAGAATCAGGGGATAACGAGGAAAGAACATGTGAGCAAAGGCC
 AGCAAAAGGCCAGGAACCGTAAAGGCCGGTTGCTGGCTTTCCATAGGC
 TCCGGCCCGCTGACGAGCATCACAAATCAGCGCTCAAGTCAGAGGTGGCGA
 ACCCGACAGGACTATAAGATACCAAGGGTTCCCCCTGGAAAGCTCCCTGTC
 GCTCTCTGCTGGCTTCCCTGCTTACCGGATACTGTCGCCCTTCTCCCTC
 GGGAGCGTGGGCTTTCTCATAGCTACGCTGAGGTATCTCAGTCGGGTAG
 GTCGTTGCTTCAAGCTGGCTGTGTCACGAAACCCCCCTGCGCCGACCG
 TGGCCTTATCCGGTAACATCTGCTTGTGAGTCAACCCGGTAAGACAGCTT
 CGCACTGGCAACGCCACTGTAACAGGATTAGCAGAGGGTATGTAGGG
 GTGCTACAGACTCTGAAAGTGGCTCAACTACGGCTACACTAGAAGAACAGT
 ATTTGGTATCTCGCCTCTGCTGAAGCCAGTTACCTTCGGAAAAGAGTTGGTAGC
 TCTTGTATCGGCAAAACAAACCCCGCTGGTAGCGGTTTTTGTGTTGCAAGC
 AGCAGATTACGCGCAGAAAAAGGATCTCAAGAAGATCCTTGATCTTCTACG
 GGGTCTGACGCTCAGTGGAAAGGAAACTCACGTTAAAGGATTGGTACAG
 TCTAAAGTATATGAGTAAACCTGGCTGACAGTACCAATGCTTAATCAGTGAG
 GCACCATCTCAGCGATCTGCTATTGCTCATAGTGGCTGACT

-continued

RAW SEQUENCE LISTING

SEQ ID NO: 25. NUCLEOTIDE SEQUENCE OF PLASMID 5297
 GCGCTATGCTCTGCCAGTGTACAACCAATTAAACCAATTCTGATTAGAAAACCTC
 ATCGAGCATCAAATGAAACTGCAATTATTATTCATACGATTATCAATACTCCATATT
 TTGAAAAAGCCGTTCTGTAATGAAAGGAAAACCTCACCGAGGCAGTTCCATAGG
 ATGGCAAGATCTGGTATCGCTCTGGACTCGTCAAATCAACAAAC
 CTATAATTCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCAGAGTG
 AGCACTGGTACCGGTAGAATGCCAAAAGCTTACGATTCTTCCAGACTTGTTC
 AACAGGGCAGCATTACGCTCGTCAAAATCACTCGCATCAACCAACCGTTA
 TTCATTGTTGATTCGCTGAGCGAGCAGAAATACCGGATCGCTTAAAGGAC
 ATTACAAACAGGAATCAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAAC
 ATATTTACCTGTAATCAGGATATTCTCTAACTCTGGAAATGCTGTTTCCC
 GGATCCGACTGGTAAACCATCGCATCACGGACTACGGATAAAATGCTTGAT
 GCTGGAAGAGGCATAAATTCGTCAGCCAGTTAGCTGACCATCTCATCTGTA
 ACATCATTGGCAACGCTACCTTGCCATGTTTCAAGAACAACTCTGGCGATCGG
 GCTTCCCATATAATCAGCATCCATGTTGAATTTAAATCGGGCCTCGAGC
 CCATTATACCCATATAATCAGCATCCATGTTGAATTTAAATCGGGCCTCGAGC
 AAGACGTTCCCGTTGAATAATGGCTCATAAACCCCCCTGTTACTGTTATGTA
 GCAGACAGGTGACAATATTGGCTATTGGCATTCGATACGTTGATCTATATCAT
 ATATGTACATTATATTGGCTCATGTCATGTCATGACCGCCATGTTGACATTGATTA
 TTGACTAGTTAAATAGTAATCAATTACGGGTCATTAGTCATAGCCCATATATG
 GAGTTCCGCTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCAAAC
 GACCCCCGCCATTGACGTCAATAATGACGTATGTTCCATAGTAACGCCAATAG
 GGACTTCCATTGACGTCATGGTGGAGTATTACGGTAAACTGCCACTTGGC
 AGTACATCAAGTGTACATATGCCAAGTGGCCCGCTTATGACGTCATGACGGT
 AAATGGCCGCTGGCATTATGCCAGTACATGACCTTACGGTATGCGGTTTGGCA
 GGCAGTACATCTACGTTAGTCATCGTATTACCATGGTATGCGGTTTGGCA
 GTACACCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCAC
 CCCATTGACGTCATGGGAGTTTGGCACCATAACGGGACTTCCCAA
 ATATGCTTAATAACCCCGCCCGTTGACGCAAATGGCGTAGGCGTGTACGGT
 GGGAGGTCTATAAAGCAGACTCGTTAGTGAACCGTCAGATCGCTGGAGAC
 GCCATCACCGCTGGCTTGTGACCTTACAGAACAGTAAACCCCTGCTCCGATA
 GCGCCGGGAAACGGCATTGGGACGGTGGCAAGAGTGAACGACTCA
 CGTCCGGATCTCAGCAAGCAGGTAGTACTCTCAGGGTGGCTGGCTTCCC
 CAGTCAGAAGACTCAGGGATTGGAGGACGCTGTTGGCTCTTCATGTA
 TTTGCTTGCCTCAACCTGACTATCTCCAGGTAGGATCCAGAGTCAGGGT
 CTGTTATTCCTGCTGGCTTGTGACCTTACGGTAACTGGGACCTTGCTGCT
 TTGCTCTCACATCTGTCATCTCCGCGAGGACTGGGACCTGTGACAAACAT
 GGCTAGATTGTTGGAGGCTGGGAGTGGCGAGAACGATTCCAACCCGGAGT
 GCTTGTGCGCTCGTGGCAGGGCAGTCTGGCGCTGTGTTGGGTGACCCCA
 GTGGGACAGCTTACAGCTGGCATTGGGACGATTGAAACAGTGGGAGTCTTGA
 CCCAAAGAAACTCAGTGTGGACCTCCATGTTATTCCATGACGTGTTG
 GCAAGTTCACCCCTCAGAGGTGACCAAGGTTCATGCTGTGTGCTGGAC
 AGGGGAAACGGACACTGCTGGGTGATTCTGGGGCCACTTGTGTTGTAATGG
 TGTGTTCAAGGTACAGCTGGGCTTAACAAAACAAAAGATGGGTTATTCC
 CTAACATTCTGGGTACGTAATTGGAAGTGGGGGACATTGCCACAAAGATCAT
 TGACAAAAGATCAAGGATTGGGCTTTGGGCTTGTGCTCCATTACACAA
 TGTTGGATATCTGCTTAATGCTTGTATGCTATACAAGCTAAACAGGTTT
 CACTTCTGCGCAACTTACAAGGCTTCTCAAGTAAACAGTACATGAACTTAC
 CGTGTGCGAACGGCTGGTCTGTGCGCAAGTGTGCTGACGCAACCCCA
 CTGGCTGGGCTGGCCATAGGCCATCAGGCCATGGTGGAAACCTTGTGCTC
 CTCTGCCGATCCATACTGCCAACCTAGCCGCTTGTGCTCGCAGCGGTC
 TGGAGCAAGGCTAGGAACCTGACAATTCTGCTGCTCTGGGAAATATACA
 TCGTTTCGATCTACGTTGATCTTTCCTCTGCGAAAGGAAATATGGGACATCAT
 GAAGCCCTTGGGCTGGCATGACTCTGGCTTAACGGGATAACCGCAGGAAAC
 TAGTGTGTTGGAAATTGGTGTCTCTACTCGGAAGGAATTCTGCTTAATGA
 CGGCCAACCGGGGGAGAGGGGTTGGCTATTGGGCGTCTCGGAAATATACA
 GCTCACTGACTCGCTCGCTCGTCTCGGCTCGCGGAGCGGTATCACTCA
 CTCAAAGGGGTAATACGGTTACCCACAGAACATCAGGGATAACCGCAGGAAAC
 ATGTGAGCAAAGGCCAGCAAAGGCCAGAACCGTAAAGGCCCGTGTG
 GCGTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAATCGACGCTAA
 GTCAGAGGTGGGAAACCCGACAGGACTATAAAGATACCGAGCTTCCCGT
 GAAGCTCCCTCGCCTCTCTGTTGCGACCTCTGCCGCTTACCGGATAACCTG
 CGCTTCTCCCTCGGAAAGCGTGGCGCTTCTCATAGTCACGCTGTAGGTAT
 CTCACTGGGTAGGTCGTTGCGCTCAAGCTGGCTGTGCGACGAACCCCG
 GTTCAGCCGACCGCTGCGCTTACGGTAATCTGCTTGTGCTGACGCTAA
 TAAGACAGCACTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
 CGAGGTATGAGGCGGTGCTACAGAGTTCTGAAAGTGGGCTAACTACGGCT
 AACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCAGTTACCTCGG
 AAAAGAGTGGTAGCTTGTACCGGAAACAAACCCGCTGGTAGCGGTGGT

- continued

RAW SEQUENCE LISTING

TTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCC
 TTGATTTCTACGGGCTGACGCTCAGTGGAACGAAAACCTACGTTAAGGG
 ATTTGGTCAGAGATTATAAAAAGGATCTCACCTAGATCCTTTAAATTAAAAA
 TGAAGTTTAAATCAATCTAAAGTATATGAGTAAACTTGGCTGACAGTACCAA
 TGCTTAATCAGTGAGGCACCTATCTAGCGATCTGCTATTGTTCACTCATAGT
 TGCTGACTC

SEQ ID NO: 26. NUCLEOTIDE SEQUENCE OF PLASMID 460
 GAATTCTGCAATTATGAATCGCCAACCGCGGGGAGAGGGGTTGCGTATTG
 GCGCTCTTCCGCTTCGCTACTGACTCGCTCGCTCGGCTCGGCTGC
 CGCAGGGTACGCTACTCAAAAGCGGTAAATACGGTTATCCACAGAACTAG
 GGATAACCCAGAGAAACATGTGAGCAAAAGGCCACGAAAGGCCAGAAC
 CTAAAAGGCCCGTGTGGCTTCCATAGGCTCCGCCCTGACGAGC
 ATCACAAAATCAGCTCAAGTCAGAGGTGGCGAACCCGACAGGACTATAAAG
 ATACAGGCTTCCCTGAGGCTCGGCTCTCTGTTCCGACCCCTG
 CGCTTACCGGATACCTGTCGCCCTTCCCTCGGAGCGTGGCTTCTC
 ATAGCTCACGCTGTAGGTATCTAGTTGGTGTAGGCTGTGCTCCAAGCTGGG
 CTGTGTGACGAACCCCCGTTAGCCGACCGCTGCGCCTATCCGTAACTA
 TCGTGTGAGCTTCCCTGAGGCTCGGCTCTCGGCTACAGGTTCTGAAG
 GTAAAGGATTAGCAGAGCGGGTATGAGGCTGCTACAGGTTCTGAAG
 TGGTGGCTAACACTAGGCTACACTAGAAGAACAGTATTGTTATCGGCTCTGC
 TGAAGCCAGTTACCTCGGAAAAGAGTTGGTAGCTCTTGATCCGGAAACAAAC
 CACCAGGGTGTGGGTTTTTGTGGCAAGCAGCAGATTACCGCAGAAAAA
 AAAGGATCTCAAGAAGATCCTTGATCTTACGGGTTCTGACGCTCAGGGA
 AGGAAACACTAGGTTGGTCAAGGATTATGAGATTATAAAAGGATCTTCACC
 TAGATCCTTTAAATTAAATGAAGTTTAAATCAATCTAAAGTATATGAGTAAA
 CTGGTGTGACGTTACCAATGCTTAATCACTGAGGCAACCTATCTCAGCGATCTGT
 CTATTCTGCTCATCCATAGTTCGCTGACTCGGCGTAATGCTCTGCCAGTGTACA
 CCAATTAAACCAATTCTGATTAGAAAACCTCATCGACATCAAATGCAATT
 ATTCAATCAGGATTATCAATACATATTGAAAAGCCGTTCTGTAATGAAGG
 AGAAAACCTCAGGAGGAGTCCATAGGATGCGCAAGATCTGGTATCGGTCTG
 GATTCCGACTCTGCAACATCAACATACACCTATTTCACCTCGTCAAACAA
 GTTATCAAGTGAGAAATCACATGAGTGAAGACTGAATCGGTGAGAATGCAA
 AAGCTATGATTCTTCCAGACTGGTCAACAGGCCAGCATTACGCTCGTC
 CAAACACTCGCATCAACCAACCGTTATCATTGCTGATTGGCCTGAGCGAG
 AGCAAAATCGCAGCTGCTGTTAAAGGACAAATACAAACAGGAATCAAATGCAAAC
 CGCGCAGGAACACTGCCAGGCCATCAACATATTTCACCTGTAATCAGGATATT
 CTTCTAATACCTGGATGCTGTTCCGGGATCGCAGTGGTAGTAAACATGC
 ATCATCAGGAGTACGGATAAAATGCTGATGCGGAAGAGGCATAAATTCCGTC
 AGCGAGTCTGCTGACCATCTCATCTGTAACATCATTGCAACGCTACCTTGCC
 ATGTTTCAGAAACACTCTGGCGCATGGGCTTCCCATAACATGATAGATTGTC
 GCACCTGATTGGCGACATTATCGCAGGCCATTATAACCCATATAACGACATC
 CATGTTGGAAATTAAATCGGCCCTCGAGCAAGACGTTCCCGTTAATATGCTC
 ATAACACCCCTGTTACTGTTATGTAAGCAGACAGGTACCAATCTTCGAGT
 GAGGAGACAAAAAAATTCCAAACACTATTGCAATGAAAATAATTCCCTTATTAG
 CCAGAAGTCAGATGCTCAAGGGCTTCATGATGTCCTCATAATTGTCAGAGG
 GAAAAGATCATCGTAGATGAAACGATGTATATTCCGAGGAGGACGACAGA
 ATGTCAGTTCTGAGCTGCTCCAGACGGCTGCGAGCAGAAACAAAGGGCT
 AGGAGTTCGCGAGTATGGATCGGCAGGGACCAAAGGTTCCACGGCATCGC
 TGATGGCTATGCCAAGCCCCAGTCAGTGGGGTTGCGTCAGCAAACACTTGG
 CACAGACCAAGGGCTGCGAGCAACGGGTTAAGGTTATGTAATGCTTACCTTA
 GAAAGGGCTTGTAGTTGGCGAGAAAGTGAAGCCTGTTAGCTTGTATACATGC
 ATACAAAGGCTTACAGGATATCCACATTGTAATGGAGCAGCAAAGCCC
 AAAAGACCCAAATCCTTGACATACTTCCAAATCAATAGGCTGTTACAGGAAG
 TTTCTAAAACAGTGGTGTGATTTGACAATATGATCTTGTGGCAATGCCCCA
 ACTTCAATTACGTAACCCATGAAGTTAGGGAAATAACCCCATTTTGTGTTGTT
 AGGGCCCAGATCTTAGGCTACTCATCAAAGTCTCTGCAGCTGCCCTGACTGT
 GAAGGCTGCAACATAATCTGCTCTTCACTTCCACGGCTTGGAGGTCC
 ACTTGTGTTCAATCAACAGAGCATCAAATTCTGGGAATGACTCCCTGC
 ATACTGTTGCTGCTGGAGCATAGATGACATGCCATAAAAAGGCTGTCT
 GTGTAACCTGTTGCTTCCGAACTGAGTGGTGTGATATTCA
 CATTCTTAATACTATTGGGTTGCTTGTCAAAGTCTGAGCTCTCACTGAACTT
 GGAAGCAATTCTGAAATTCTTACTGCAAGAAAAGTGAATCAAATGATACAC
 TGATGTTCTTACTGAGTGTGTTGATAGAATCTGAGTTTGTGAGCAT
 ACTTCTTAAACAGCATATACTCGACAATAACCGCAACTATGAAATTG
 GCCAGCTCAAACACCATCCCTCGAACCTGGGCCACAGTGAGGTGATATTAA
 ACATTGGATCATAAAACCTTCCACCAACTCATATGTTCATAGACACTGTGATACA
 GTGGATGCCGCTGAAATTGTTGTTCCAAATTGTTAGTATACCGTCTGCT
 GAAGCAATTCCAGTCGTTGGAGAACACCTCAAATCTGGGAATGACTCC
 GCTTATCTGGGCTGCAACTGGAACCTGAAACTCTGGGAAGGACTTTTGTGTT
 CATAAAAGAGATTGCTTCAAGGCTTCACTGGGCTTTCAAGCTTGTGTTAGG
 TTGTTGACCAAGCTGACATCAGCGGTGTAACATCAACTCTCAGAGTGTAGTT
 TTCTATAGATGAGTCAGCATTAAATAAGGCCACGCCAGCTTGTGAGGAGTCTG
 ATTCTCTGCCCCACTCAGTAGAACCAAGAACACCAATTCTCTGATCCAG
 CTGCAACAAAATTGTTCTCTAGGTCTCCACCCCTCTTTCAGTGTGTTCAAAG
 CTCTCACAATTCTGAAACACAGCTGCCACTCTGAGGGTCAATACCACCAA
 CACCCATGAGTCCCGTGAACCTCCAGAAATGACATATCTGCTGGTTACTGCT

- continued

RAW SEQUENCE LISTING

CCTCTGAGAGTACCTATCACATTGAAATTCTGTCACTTCATTGGTAGAGTGGAT
 GTGCATCTTGACTTTTGTAAGAAAAGTTCCAGTAAAGCAGGTCACATTGT
 AGGGCACTTGGACTTCCCTCCAGCTGCTATCTGGTGTCTGAGCCACCAT
 TTTTCTAGGAGCTCTGTGCACTAGTATCAATTGGATGAACAGGAATACTTG
 GAAGACCAACACGCCCTGCAATTCCACGCCATAAGCATATTCACTTGCTGGAA
 CCTGGTGTGAGAGGGTCTCGCACCATCAGATTAGGATATTCCACGCTGGA
 CACCACCTCCAGGAAGATTCCAACCATCTGGATAAGGACTTCACCCCCAGGAGCAA
 GTAGTCAGCAGGGCTGGAGTAGAGAATGACTCTTGGCCCTGCCAGCTGGC
 ATTCTTAACCTTATTCTGAAACATTTCACATCTGGCAATTACAATTTC
 GAGCAATTGATTTCATGTCGGTCAATTAAAGAAGTCTCTAGTCGTCATA
 GTAACTACATACAGTACGGCATTCTGGAGAGAGAAAGCAGTCAA
 GTGGTACAATACTCGAACATTTCATATCTGGAGGAGGGTCAATTATGA
 TGTGTTGAAAATCTCATTCATCTGATATTGAGATGTTGAGGATGAGT
 CTTATTGGTAGGACAACAGGACATCATAATGTGCCAGCTAACAGAACTCAGG
 CCAAATCTTCCACTGGGATGAAATTGCTTGAAGCTGAAAGTTTGTCTGTT
 CCTGCTAAATGTGTATCTGTGTAATTATAAGAACCTCTGTCTCAGCT
 TCAATTCTACCAAAATGCTTCATATTATGCTTGGAGTAATGTTAGTC
 TTGGAGGATTATAACCACCGAAGAGGAAGGCCAGGGAGAAAGAACCC
 GCCAGCACCGGCCACAGGCCACAGCCAGGGCGGGCGGCTAGCCA
 TGTCGTCACAGGGCTCCAGTCTGGAGGATTGACGAGATGTGAGAGGCAA
 TATTGGAGCAGGGTTACTGTCTGAACTGGACCCACAGCAGGAAATACAG
 ACCCTGACTCTGGATCCTGACCTGGAAGATAGTCAGGGTTGAGGAAGCAA
 AGGTACATGTAAGGAAGAGGCCACAGCGTCCCAAATCTGGAGTCTGACT
 GGGAAAGCAGGGCACCTGGAGGATTCACCTGCTTGTGAGATCCGGACG
 GTGAGTCACTCTGGCACGGGAATCCGGTTCACATGCAACGGTCCCGCC
 GGAGGCTGGATCGGTCCCGGTCTCTATGGAGGTCAAAACAGCGTGGATGGC
 GTCTCAGGGATCTGAGGTTCAACTAACAGGCTCTGCTTATATAGACCTCCA
 CGTACACGCCATTGGCCATTGGCTCAACGGGGGGGTTATTACGACATT
 GGGAAAGTCCCGTTGATTGGTGTGACCTGAGGTACCAATTGGCTATTG
 GCCATTGCACTGGTGTATCTATATCATATAATGTACATTATATTGGCTCATGTC
 ATATGACCGCATGTTGACATTGAAATTGACTGTTATTAAAGTAATCAATTAC
 GGGGTACTTGGTGTGACCCATATGGAGGTTACATAACTACCGTAA
 ATGGCCCGCCCTGGCTGACGCCAACGACCCCGCCATTGACGTCATAATGA
 CGTATGTCCTAGTAACGCCATTGGACTTCCATTGACGTCATGGTGG
 GTATTTCAGGTAACCTGCCACTTGCAGTCATCAAGGTATCATATGCCAAGTC
 CGCCCTTATTGACGTCATGACGGTAAATGGCCCGCTGGCATTATGCCAGTA
 CATGACCTTACGGGACTTCTCTACTTGGCAGTCATCTACGTTATTAGTC
 TTACCATGGTGTGCGTTTGGCAGTACACCAATGGCGTGGATAGCGGTTGA
 CTACGGGGATTCCAAGTCTCCACCCATTGACGTCATGGAGTTGTTGG
 CACCAAAATCAACGGGACTTCCAAATGTCGTAATAACCCCGCCCGTGGACGC
 AAATGGCGGTAGGGCTGTACGGTGGAGGTTATATAAGCAGAGCTGTTAG
 TGACCGTCAGATGCCCTGGAGACGCCATTACGCTGTTGACCTCATAGAAG
 ACACCGGGACCGATCAGCCTCCGGCCGGAACCGGTGATGGAACCGCGA
 TTCCCGTGCAGAGTGAACCTACCCCTCCGGATCTCAGCAAGCAGGTATGACTC
 TCCAGGGGGGGCTGGCTTCCCAGTCAGTCAGACCTCCAGGGATTGAGGGACGCTG
 TGGGCTCTCTTACATGTTACCTTGTCTGCCCTAACCTGACTATCTCAGGA
 TCAGGATCCCAGAGTCAGGGCTGTGTTCTCTGTCGTCGTCATCTCCGGAGGA
 ACAGTACGGCTCTGGCAATGGCTGACGGTGGAGGTTATGAGCTGGCAGTC
 CTGGGGACCTGTGACGAACTGGCTAGCAAGGGCTGTGCTTGCCTGTTGA
 TGGCAGGCTTGGCCCTGCAAGGACTGCCCTGCTGTACTCTGCAAAG
 CCCAGGTGAGCAACGAGGACTGCCCTGCAAGGGTGGAGAACACTGCACCCAGCTGGG
 GAGCAGTGTGACCCGGCGCATCCGGCAACTTGGCTCTGACGGCTCATCAGC
 AAAGGCTGAGCTTGAACACTGCGTGGAGTACACAGGACTACTACGGGCAAG
 AGAAACATCACCTGCTGTGACACCGACTTGTGCAACGCCAGGGGGCATGCC
 CTGCAAGCGGCTGCCCATCTTGCCTGCTCCCTGCACTCGGCTGCTGTC
 TGGGGACCCGGCAGCTAGAGATCTGGGCTAACAAAACAAAAGATGGG
 TTATTCCTTAAACTTCTGTTGATGGTAAATTGGAAGTTGGGGACATTGCCACAG
 ATCATATTGTAACAAAGATCAACACTGTTTAAAGAAAATCTGCTAAACAGGCC
 TTGATGGAAAGTATGTCAAAGGATTGTTGGCTTGTGCTCCATT
 ACACATGTGGATATCTGCTTAATGCTTGTGATGCACTGATAACAGCTAAACA
 GGCTTCCACTTCTCGGCAACTAACAGGCTTCTAAGTAAACAGTACATGAAAC
 TTACCCCTGCTGCGCAAGGCCCTGGCTGTGCTGCAAGTGTGCTGACGCCAC
 CCCACTGGCTGGGCTGGCATAGGCCATCAGCGCATGCGTGGAAACCTTGT
 GCTCTCTGCGGATCCATCTGCGGAACTCTAGCCGCTTGTGCTCTCGCGAGC
 CGCTCTGGAGCAAGGCTCATAGGAACCTGACAATTCTGCGTCTCTCGCGAAAT
 ATGAGATCTGGTATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
 ATCATGAAGCCCTTGAGCATCTGACTCTGCTAAATAAGGAAATTATGGGAC
 GCAATAGTGTGTTGAAATTGGTGTCTCACTCGGAAGC

SEQ ID NO: 27. NUCLEOTIDE SEQUENCE OF PLASMID 451
 GGCGTAATGCTGCCAGTGTACAACCAATTAAACCAATTCTGATTAGAAAAACTC
 ATCGAGCATAAATGAAACTGCAATTATTATCATATCAGGATTATCAACCATATT
 TTGAAAAGCCGTTCTGTAATGAGGAGAAAACCTACCGAGGGCAGTCCATAGG
 ATGGCAAGATCTGGTATCGCTGCCGATTCCGACTCGTCCACATCAACAC
 CTATTAAATTCCCTGTCAAAATAAGGTTATCAAGTGAGAAATCACCAGTGGATG
 ACGACTGAATCCGGTGGAGAATGGCAAAGCTTATGCAATTCTCCAGACTTGGTC
 AACAGGGCAGGCCATTACGCTGTCATCAACCAACTCGCATCAACCAACCGTTA

- continued

RAW SEQUENCE LISTING

TTCATTCTGATTGCGCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGAC
 AATTACAACAGGAATCAAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAAC
 AATATTTCACCTGAATCAGGATATTCTCTAATACCTGGAAATGCTGTTTCCCG
 GGATCGCAGTGGTGAATAACCAGTCATCAGGAGTACGGATAAAATGCTTGAT
 GTCGAAAGGGCATAAATTGGTCACTGGGAGTTAGTGTACGGCATCTCATCTGTA
 ACATCATTGGCAACGCTACCTTGCCATGTTAGAAACAACCTGGCGCATCGG
 GTTCCCATAACATCAGTAGATGTCGGCACCTGGATTTCCCG
 CCATTATACCCATAACATCAGCATCTGGAAATTAAATCGGGCCTCGAGC
 AAAGACGTTCCGGTTGAATAATGGCTCATACACCCCTTGATTAATCTGTTATGTA
 GCAGACAGGTGACAATATTGGCTATTGGGATTGACATACGTGTATCATATCAT
 ATATGTCAATTATATTGGCTCATGTCCATATGACCGCCATTGACATTGATTA
 TTGACTAGTTAAATAGTAATCAATTACGGGCTATTAGTTCATAGCCCATAATAG
 GAGTCCCGGTTACATAACTACGGTAAATGGCCCGCTGGCTGACCGCCAAC
 GACCCCCGCCATTGACGTCAATAATGACGTATGTTCCATAGTAACGCCAATAG
 GGACTTCCATGACGTCATGGGTGGAGTTACGGTAAACTGCCACTTGGC
 AGTACATCAAGTGTATCATATGCCAAGTCCGGCCCTTATTGACGTCAATGACGGT
 AAATGGCCGCGCTGGCATTAATGCCAGTACATGACCTTACGGACTTCTACTT
 GGCAGTACATCTACGTTAGTCATCGTATTACCATGGTATGCGGTTTGGCA
 GTACACCAATGGCGTGGATAGCGGTTTGTACTCACGGGATTTCAGTCTCCAC
 CCCATTGACGTCAATGGGAGTTGGCACCATAACCGGACTTCCAA
 ATGTCGTAATAACCCGCCCGCTTGAACGAAATGGCGTAGGGTGTACGGT
 GGGAGGTCTATAAAGCAGACTGTTAGTGAACCGTCAGATCGCTGGAGAC
 GGCATCCACGCTTTTGACCTTCAATAGAACGACACCGGACCGATCCAGCTCC
 GCGGCGGAAACGGCATTGGAAACGGGATTCCCGTGCACAGAGTGA
 CGTCCGGATCTCAGCAAGCAGGTATGACTCTCAGGGTGGCTGGCTTCCC
 CAGTCAGACTCAGGGATTGAGGAGCCTGTTGGCTCTCTTACATGTAC
 TTTGCTTGCTCAACCTGACTATCTCAGGTAGGATCCCAGAGTCAGGGT
 CTGTAATTCTCTGGCTTCAAGTCAACAGTAAACCTCTGGCAATA
 TTGGCTCTCACATCTCGTCACTCCGGCGAGACTGGGACCTGTGACAAACAT
 GGCTAGCGCGCCGCCGCGCTGGCTGTGCGTGGCGCTGGTGTGGCG
 GTGGATTCTCTCGCTCCCTGGCTTCTGGTGGTTATAAAATCCTCCAATGA
 AGCTACTAACATTACTCCAAAGCATAATATGAAAGCATTGGATGAATTGAAAG
 CTGAGAACATCAAGAATTCTTATAATTTCACAGATAACCAATTAGCAGGA
 ACAGAACAAAACCTTCAGCTGCAAGCAAATTCAATCCAGTGGAAAGAATTG
 CCTGGATTCTGTGAGCTGGCACATTATGATGTCCTGTTGTCCTACCCAAATAAGA
 CTCATCCCAACTACATCTCAATTAAATGAAAGATGGAAATAGGATTTCAACACAT
 CATTATTGAAACACTCCTCCAGGATATGAAATGTTGAGTATTGACCA
 TTCAGTGTTCTCTCTCAAGGAATGCCAGAGGGCGATCTAGTGTATGTA
 TGACGAACACTGAAGACTTCTTAATTGAAACGGGACATGAAATTCAATTGCTCTG
 GAAAAATTGAAATTGGCAGATGGAAAGTTTCAAGAGGAAATAAGGTTAAAAT
 GGCAGCTGGCAGGGGAAAGGAGTCATTCTCACTCCGACCCCTGCTGACTAC
 TTGCTCTGGGTGAAGTCATTCAGATGGTGGAAATCTCTGGAGGTGGT
 TCCAGCGTGGAAATATCTTAATCTGAATGTCAGGGAGCCTCTCACACCA
 TTACCCAGCAATGAATATGCTTATAGGCGTGGAAATTGAGGGCTGTTGCTT
 CCAAGTATTCTGTTCATCCAATTGGATAATTGATGCAAGAACGCTCTAGAAAA
 ATGGGTGGCTCAGCACCCAGATAGCAGCTGGAGAGGAAGTCTAAAGTGC
 CTACATGTTGGACCTGGCTTACTGAAACTTTTACACAAAAAGTCAGATGC
 ACATCCACTACCAATGAAGTGAACAATTACAATGTAAGGTACTCTCAGA
 GGAGCAGTGGAAACAGACATGTCATTGGAGGTACCCGGACTATGG
 GTGTTGGTGTATTGACCTTCAGACTGGAGCAGCTGTTGTCATGAAATTGTA
 GGAGCTTGGAAACACTGAAAAGGAAGGGTGGAGACCTAGAAGAACATTGTT
 TCGAAGTGGAGAAGTGGCTTCTTGTTCTACTGAAGTGGCAGAG
 GAGAATTGAAAGACTCCTCAAGAGCGTGGCTTATATTAAATGTCAGTCA
 CTATAGAAGGAAACTACACTTGAGAGTTGATGACACCCGCTGATGTCAGC
 GGTACACAAACCTAACAAAGAGCTGAAAAGCCCTGATGAAGGCTTGAAGGAAA
 TCTCTTATGAAAGTGGACTAAAAAAAGTCTTCCCGAGGTTGAGTGGCATGCC
 CAGGATAAGCAATTGGGATCTGGAAATGATTGAGGTGTTCTCCAAACGACTTG
 GAATTGCTTCAGGCAGAGCACGGTAACTACAAAAATTGGAAACAAACAAATTCA
 CGGCTATCCACTGTATCACAGTGTATGAAACATATGAGTTGGTGGAAAAGTTT
 ATGATCCAATTTAAATATCACCTCACTGTGGCCAGGTTGAGGGATGGT
 GTTGGAGCTGGCCATTCTGGCTTCTGGATGTCAGGATTATGCTGTTG
 TTTAAAGAAAGTATGTCAGAAATTCTACAGTATTCTATGAAACATCCACAGGAAA
 TGAAGACATACAGTGTATCATTTGATTCACTTTCTGAGTAAAGAACATTGAC
 AAATTGCTTCAAGTCACTGAGAGACTCCAGGACTTTGACAAAAGCAACCCAAATA
 GTTAAAGAATGATGATCAACTCATGTTCTGGAAAGAGCATTATGATCC
 ATTAGGGTTACACAGGGCCTTCTGATGGCAGGTTGATGGCAGGATTATGCTG
 CACAACAGTATGCAAGGGACTCATTCCAGGAAATTATGATGCTCTGTTG
 TGAAAGCAAGTGGACCTTCAAGGCCCTGGGAGAGTGAAGAGACAGATTAT
 GTGCACTGGCTTACAGTGCAGGCACTGGAGACTTTGAGTGAAGTGGCGA
 TCCGAAGGTAGGGGTTATTGACCTGTGGAGATGTCGAAGAAAACCCAGGAC
 CCGCAAGCAAGGCTGTGCTGTCCTGGCCCTGTTGATGGCAGGCTTGGCCCTG
 CAGGCACTGCCCTGCTGTGCTACTCTGCAAGGCCAGGTGAGCAACGAGGACT
 GCGCTGAGGTGGAGAACTGCACCCAGCTGGGGAGCAGTGCCTGGACCGCGC
 ATCCGGCAGTGGCTCTGACCGTCATCAGCAAGGCTGCAGCTGTA
 GTGGATGACTCACAGGACTACTACGTGGCAAGAAGAACATCACGTGCTG
 ACCGACTTGTGCAACGCCAGCGGGCCCATGCCCTGCAAGCCGCTGCC
 CCTTGCGCTGCTCCCTGCACTGGCGCTGCTGGGGACCCGGCAGCTATA

- continued

RAW SEQUENCE LISTING

GAGATCTGGGCCCTAACAAAACAAAAAGATGGGGTATTCCCTAAACTCATGGG
 TTACGTAATTGGAAAGTGGGGACATTGCCAACAGATCATATTGTACAAAAGATCA
 AACACTGTTTAGAAAACCTCTGTAAACAGGCCATTGATTGGAAAGTATGTCAA
 AGGATTGCGGTCTTGGCTTGCTGCCATTACACAATGTGGATATCCTGC
 CTTAATGCCCTTGATGCATATAAAGCTAACAGGTTTCACTTCTGCCAA
 CTTACAAGGCCCTTCAAGTAAACAGTACATGAACCTTACCCGTTGCTGCCAA
 CGGCCCTGCTGTGCAAGTGTGCTGACGCCAACCCCCACTGGCTGGGCTTG
 GCCATAGGCCATAGCGCATGGCTGGAAACCTTGTGGCTCCTGCCGATCCATA
 CTGGAAACTCTAGGCCCTGGTTGTCTGCCAGGGCTGGAGCAAAGCTCAT
 AGGAAGTACAAATTGTGCTCTCGCAGAAATAACATCGTTGATCTACGT
 ATGATCTTCTCTGCAAAAGGAAATTATTCATTGCAATAGTGTGTTGAAATT
 TTGCTGCTCTCACTCGAAGGAATTCTGCAATTATGAATCGCCAACGCCGGGG
 AGAGCGGTTGCGTATGGCGCTCTCCGCTCTCGCTACTGACTCGCTG
 CGCTCGTGTGCGTGGCGAGCGGTACTAGCTCAACTCAAGGGCGTAATA
 CGGTATCCACAGAACGGGATAACCGAGAAAGAACATGTGAGCAAAGGC
 CAGCAAAGGCCAGAACCGTAAAAGGCCGTTGCTGGGTTTCCATAGG
 CTCCGCCCTGACAGCATCACAAATCGACGCTCAAGTCAGAGGTGGCA
 AACCCGACAGGACTAAAGATACAGGCGTTCCCGCTGGAGCTCCCTCGTGC
 GCTCTCTGTCGACCCCTGCCGCTTACCGGATACCTGTCGCCCTTCCCTC
 GGAAGGGTGGCTTCTCATAGCTCACGCTGTAGGTATCTAGTCGGTTGAG
 GTCTGCTGCCAAGCTGGCTGTGACGAACCCCCGTTGAGGCCGACCGC
 TCGCCCTATCGGTAACAGGCTTCCGAGGAGTGGCTGGGTTTTGAGC
 CGCTCTGAGCTGAGCTGGCTGGAGGAGTGGCTGGGTTTTGAGC
 ATTTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGGAAAAGAGTTGGTAGC
 TCTGATCCGCAAACAAACACCCTGCTGGTAGCGGGTTTTGAGC
 AGCAGATTACGCCAAAGGATCTCAAGAAGATCTTGTATTTCTAG
 GGCTGACGCTCAGTGAACGAAACTCAGTTAAGGGATTGGTCTAGAGAT
 TATCAAAAGGATCTCACCTAGATCCTTTAAATTAAAATGAAGTTAAATCAA
 TCTAAAGTATATATGAGTAAACTTGTCTGACAGTACCAATGCTTAATCAGTGAG
 GCACCATCTCAGCGATCTGCTATTCTGCTCATAGTGGCTGACTC

SEQ ID NO: 28. NUCLEOTIDE SEQUENCE OF PLASMID 454
 GCGTAATGCTGCGAGTGTACACCAATTACCAATTCTGATTAGAAAAACTC
 ATCGACATCAATGAAACTGCAATTATCATATCAGGATTATCAATACCATATT
 TTGAAAAGCGCTTCTGTAATGAAAGGAAACTCACCGAGGCAGTCCATAGG
 ATGGCAAGATCTGGTATCGCTGCGATTCCGACTCGTCAACATCAAC
 CTATTAACTTCCCTCGTCAAAAATAAGGTATCAAGTGAAGAAATCACCATGAGT
 ACAGCTGAACTCCGGTGAGAATGGCAAAGGCTATGCAATTCTCCAGACTTGT
 ACAGGCAAGGCCATTACGCTCATCAAAATCACTCGCATCAACCAACCGTTA
 TTCATTGCTGATGCGCTGAGCGAGCGAAATACGCGATCGTAAAGGAC
 AATTCAAAACAGGAATCAAATGCAACCCGGCAGGAACACTGCCAGCGATCAAC
 AATATTTCACCTGAACTCAGGATACTCTCTAATACCTGGAATGTTTCCCG
 GGATCGCAGTGGAGTAACCTGCAATCAGGAGTACGGATAAAATGCTTGAT
 GTCAGGAAAGGCCATAAATTCCGTCAGGCAATTAGTCTGACCATCTCATGTA
 ACATCATTGGCAACGCTACCTTGCCATGTTAGAAACACAACTCTGGCGATCGG
 GCTTACATGAGATGCTGACCCGATTTATCGCGAGC
 CCTTTATACCCATAATCAGCATCATGTTGAAATTAAATCGGGCTCGAGC
 AAAGACCTTCCGTTGAATATGGCTATAACACCCCTTGATTACTGTTATGTA
 GCAGACAGGTGACAATATTGGCTATTGGCATTGCGATACGTTGATCTATATCAT
 ATATGTCACATTATGGCTCATGTCATGTTGACCGCCATTGTCGACATTGATTA
 TTGACTAGTTAAATAGTAATCAATTACGGGCTATTGTCATAGCCCCATATG
 GAGTTCCGCTTACACTACGGTAAATGGCCCGCTGGCTGACCGCCAAC
 GACCCCGCCCATGACGTAAATGACGTATGTTCCATAGTAACGCCAATAG
 GGACTTCCATGACGCTCAATGGGTGGAGTATTACGGTAAACTGCCACTTGGC
 AGTACATCAAGTGTATCATATGCCAAGTCCGCCCTATTGACGTCAATGAGGT
 AAATGGCCGCTGGCATTATGCCAGTACATGACCTTACGGACTTCTACTT
 GGCAGTACATCTACGTTAGTCATCGTATTACCATGGTGTGCGGTTTGGCA
 GTACACCAATGGCTGGAGTACCGGTTGACTCACGGGATTTCAGTCTCCAC
 CCCATTGGACGCTCAATGGGAGTTTGGCAGGAAACATCAACGGGACTTCCAA
 ATGTCGTAATAACCCGCCCGCTTACGGCAATGGCGTAGGGCTGAGGT
 GGGAGGTCTATAAAGCAGAGCTGTTAGTGAACCGTCAAGTCGCTGGAGAC
 GCCATCCACGCTGTTTGACCTTACAGAAGACACCCGGACCGATCCAGCTCC
 GCGCGCGGAGCGGTGCAATTGGAAACCGGGATTCCCGTGCAAGAGTGACTCA
 CGCTGGCGATCTCGCAAGCAGGATGTTGACTCTCCAGGGTGGGCTGGCTTCCC
 CAGTCAGACTCCAGGGATTGGAGGACGCTGTGGCTTCTCTTACATGTA
 TTTGCTGCTCAACCTGACTATCTCCAGGTCAGGATCCCAGAGTCAGGGT
 CTGTTATTCCTGCTGGCTCCAGTTCAAGAACAGTAAACCCCTGCTCCGAATA
 TTGCTCTCACATCTCGTCATCTCCGAGGACTGGGACCTGTGACGAACAT
 GGCTAGCAAGGCTGCTGCTGGCTTGGCTGGGCTGGCTGGCTGGAGGCC
 AGGCACCTGCCCTGCTGTGCTACTCTGCAAAAGCCAGGTGAGCAACGAGGACTG
 CCTGCAGGGAGAAACTGCACCCAGCTGGGGAGCAGTGTGTCAGGCCGCGCA
 TCCGCGCAGTGGCCTCTGACCGTCACTGCCAAAGGCTGCAAGCTGACTGCG
 TGGATGACTCACAGGACTACTACGTGGCAAGAAGAACATCACGTGCTGTGACAC
 CGACTGTGCAACGCCAGCGGGCCCATGCCCTGCAAGCCGGCTGCCGACATCC
 TTGCGCTGCTCCCTGCACTGCCCTGCTGCTGGGACCCGGCCAGTAGGAT

- continued

RAW SEQUENCE LISTING

CCAGACCCCTGAACCTTGATCTGCTGAAACTGGCAGCGATGTGGAAAGCAACC
 CAGGCCAATGGCAAGCGCGCCGCGCTGGCTGTGCGCTGGGGCGCT
 GTGCTGGCGGGTGGCTCTCTCTCGGCTCTCTCGGGTGGTTATAAAA
 TCCCTCAATGAAGCTACTAACATTACTCCAAGCATAATATGAAAGCATTTGGAT
 GAATTGAAAGCTGAGACATCAAGAAGTTATATAATTACAGACATCACAT
 TTAGCAGGAACAGAACAAAACCTTCAGCTTGCAAAGCAAATTCAATCCAGTGGAA
 AGAATTGGCTGGGATCTGTGAGCTGGCACATTATGATGTCCTGTTGCTTACCC
 CAAATAAGACTCATCCAACTACATCTCAATAATTAAATGAAGATGAAATGAGATT
 TCAACACATCATTTGAACCACTCTCCTCAGGATATGAAATGTTGGATATT
 GTACCACCTTCAGTGCCTCTCTCAAGGAATGCCAGAGGGCAGATCTAGTGT
 ATGTTAACATCTCAGAACACTGAAGACTTCTAAATTGGAACGGGACATGAAATC
 AATTGCTCTGGGAAATTGTAATTGCCAGATATGGGAAAGTTTCAGAGGAATAA
 GTTAAAAATGCCAGCTGGCAGGGGCAAAGGAGTCATTCTACTCCACCC
 GCTGACTACTTGTCTGGGTGAAGTCCTATCCAGATGGTGGAAATCTCTG
 GAGGTGGTGTCCAGCTGGAAATATCTAAATCTGAATGGTGCAGGGAGCCCT
 CACACAGGTTACCCAGCAAATGAATATGCTTATAGGCGTGGAAATTGAGGCT
 GTTGGCTTCCAGTATTCTGTCATCCAATTGATACTATGATGCAACAGAGCT
 CCTAGAAAAATGGGGCTCAGCACCAACAGATAGCAGCTGGAGAGGAAGCT
 CAAAGTCCCATACTGGGACCTGGCTTACTGAAACTTTCTACACAAAAG
 TCAAGATGCACTCTACCAATGAAGTACAAGAATTACATGATAGTAGGT
 ACTCTAGAGGGCAGTGGAACAGACAGATATGTCATTCTGGAGGTCAACCG
 GACTCATGGGTGTTGGTGTATTGACCCCTCAGAGTGGAGCAGCTGTTGTCATG
 AAATTGTCAGGGACTTGGGAAACTGAAGAAGGAGGAGGAGCTAGAAC
 AATTGGTGTGCAAGCTGGGATGAGAAGATTTGGCTTCTGGTCTACTGAGT
 GGGCAGAGGAATTCAAGACTCTCAAGAGCGTGGCTGGCTTATATTATG
 TGACTCATCTAGAAGGAACACTCTGAGAGTTGATTGACACCGCTGATGT
 ACAGCTGGTACACACCTAACAAAGAGCTGAAAAGCCTGTATGAGGCTTGA
 AGGCAATTCTTATGAAAGTGGCAATTAAAAGGAGTCTTCCCAGAGTTCAGT
 GCATGCCAGGATAAGCAAATTGGGATCTGAAATGATTGAGGTGTTCTCCA
 ACGACTTGGAAUTGCTTCAGGAGACGGTATACTAAAATTGGGAAACAAAC
 AAATTAGCGGCTATCCACTGTATCACAGTCTATGAAACATATGAGTTGGTGG
 AAAGTGGTTATGAGCTGGCAATTCTCATAGTGCCTCTTGTGATTGTCAGATT
 GCTGTAGTTAGAAAAGTGTGACAAATCTACAGTATTCTATGAAACATCCA
 CAGGAATGAAGACATACAGTGTATCTTGTACTTTTCTGCACTGAAAGAAT
 TTACAGGAAATCTTCAAGCTGGCTTACTGAGGACTCCAGGACTTGTGACAAAAG
 CCCAATGTTAAAGATGATGATCACTCTGTTCTGGAAAGAGCATT
 TTGATCATTAGGGTACAGACAGGCCCTTTTATAGGCATGTCATCTGCTCCA
 AGCAGCCACAAAGTATGCAAGGGAGTCATCTCAGGAATTATGATGCTCTGT
 TTGATATTGAAAGCTGGGACCCCTCAAGGGCTGGGAGAAGTGAAGAGACA
 GATTATGTTGCAAGCTTCAGAGTTCAGTGGAGACTCCAGGACTTGTGAGGTA
 GCCTAAAGATCTGGGCTAACAAACAAAAGATGGGGTATTCTCTAAACTTC
 TGGGTACGTAATTGGAAGTGGGGACATGCCACAAGATCATATTGTCACAAA
 GATCAACACTTTAGAAAACCTCTGTAAACAGGCATTGATTGAAAGTAT
 GTCAAAGGATTGGCTTGGCTCTTGCTGCTCATTACAAATGTGGATAT
 CCTGCTTAATGCCCTTGATGCTATCAAGCTAACAGGCTTCACTTCTC
 GCCAACTTACAAGGCTTCTAAGTAAACAGTACATGAACTTACCCGTTGCTC
 GCAACCGCCATGGCTGTGCAAGTGGCTGACGGCAACCCCCACTGGCTGG
 GCTGGGCTAGGCCATCAGGCCATGGGAAACCTTGTGGCTCTGCCGA
 TCCATACTGCCAACTCTAGCCCTGTTGCTGCGAGCGGCTGGAGCAA
 GCTCATAGGAACGTACAATTCTGCTGCTCTCGCGGAAATACATGTTGCT
 CTACGTTGACTCTGGCTAAAGGAAATTATGGGACATCATGAACCCCTT
 GAGCATCTGGCTCTGCGGAACTTCTGCTTAAAGGAAATTATGGGACAT
 GATTTTTGTTGCTCTGCGGAACTTCTGCTTAAAGGAAATTATGGGACAT
 GCGGGAGAGGGCTTGCATGGCGCTCTCGCTCTCGCTACTGAC
 TCGCTGGCTCTGGCTGGCTGGCGAGGGTACAGTCAGTCAGTCAGGCG
 GTAATACGGTTATCCACAGAATCAGGGGATAACCGAGGAAAGAACATGTGAGCAA
 AAGGCCAGCAAAGGCCAGAACCGTAAAGGCCCGTGTGCGTGGCTTTC
 ATAGGCTCGCCCCCTGACGAGCATCACAAATGACGCTCAAGTCAAG
 GCGGAAACCCGACAGGACTAAAGATACAGGCTTGGGAGCTCC
 TCGTGCCTCTGGCTGGCTGGCGTACGGGATACCTGTCGGCTTCT
 CCTCTGGGAAGCGTGGCGTTCTCATAGTCAGCTGAGGTTATCTCA
 GTGAGGTTGCTGGCTCCAGTGGCTGGGAGCTGGAGGTTCT
 GACCGCTGGCTTATCGGTAACTATGCTCTGAGCTAACCCGTTAGACAG
 ACTTATGCCACAGGCTGAGCAGCAGTCAGGTTAGGATTAGCAGAGCGAG
 TAGCGGCTGCTAGAGTCTGAGGTTGGCTAACACTGGCTACAGTGAAG
 AACAGTATTGGTATCTGCGCTGCTGAGCAGCTACCTTCGAAAGAGTT
 GGTAGCTTGTGATCCGGAAACAAACACCCTGGCTGGTAGCGGGTTTT
 GCAAGCAGCAGATTACCGCGAGAAAAAGGATCTCAAGAAGATCTT
 ATGAGATTATCAAAGGATCTCACCTAGATCCCTTTAAATAAAAATGAAG
 AATCAATCTAAAGTATATAATGAGTAAACTTGAGCTGACAGTACCA
 ATGAGGACACCTATCTAGCGATCTGCTATTGCTCATCCATAGTGCCT
 GACT

SEQ ID NO: 29. NUCLEOTIDE SEQUENCE OF PLASMID 5300
 GGCGTAATGCTGCCAGTGTACAACCAATTACCAATTCTGATTGAAAAACTC
 ATCGAGCATCAAATGAAACTGCAATTATTATCAGGATTCAACCATATT

- continued

RAW SEQUENCE LISTING

TTGAAAAAGCCGTTCTGTAATGAAGGAGAAAACCAACCGAGGCAGTTCATAGG
 ATGGCAAGATCCTGGTATCGCTCGGACTCGTCCACATCAATAACAC
 CTATTAACTTCCCGTCAAAAATAAAGGTTATCAAGTGAGAAATCACCAGTGAAGT
 ACGACTGAATCCGGTGAAGATGGCAAAGCTTATGCATTCTTCAGACTTGTTC
 AACAGGCAGGCCATTACGCTCGTCACTCAAAATCACTCGCATCAACCAACCGTTA
 TTCATTCTGATTGCGCTGAGCGAGACGAAATACGCGATCGCTGTTAAAGGAC
 AATTACAAACAGGAATCAAATGCAACCGGGCAGGAACACTGCCAGCGCATCAAC
 AATATTTCACCTGAATCAGGATATTCTCTAATACCTGGAATGCTGTTTCCC
 GGATCGCAGTGGTGAATCAGGATCATCGGAGTACGGATAAAATGCTTGAT
 GGTGCGGAAGAGGCATAAAATTCCGTAGCCAGTTAGTCTGACCATCTCATCTGTA
 ACATCATGGCAACGCTACCTTGTGAGAAACAAACTCTGGCGCATCGG
 GCTTCCCATAACATCGATAGATTTGCGCATGTTGAGAAACACTTGTGTTATGTA
 CCATTATACCCATATAAATCAGCATCCATGTGGAATTAAATCGGGCCTCGAGC
 AAGACGTTCCCGTGAATATGGCTCATAAACCCCCCTGTATTAATCTGTTATGTA
 GCAGACAGGTCACAATATTGGCTATTGGCCATTGCGCATACGTTGATCTATCAT
 AATATGTAATTTATATTGGCTCATGCGCATATGACCGCCATGTTGACATTGATTA
 TTGACTAGTTATAATAGTAATCAATTACGGGGTATTAGTTCATAGCCATATG
 GAGTTCCCGTACATAACTACGGTAAATGGCCGCCTGGCTGACGCCAAC
 GACCCCGCCCTTACGCTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
 GGACTTCCATTGACGCTAATGGGTGGAGTTACGGTAAACTGCCCACTTGGC
 AGTACATCAAGTGTATCATGCCAAGTCCGCCCTATTGACGCTAATGACGGT
 AAATGGCCGCCTGGATTAGCCAGTACATGACCTTACGGGACTTCTACTT
 GCGAGTACATCACGTTAGTACGCTTACCATGGTGTGCGGTTTGCA
 GTACACCAATGGCGTGGGATAGCGGTTGACTCACGGGATTTCAGTCC
 CCCATTGACGTCATGGAGTTGTTGGCACCRAAAATCACGGGACTTCC
 AATGTCGTAATAACCCGCCCGTGTACGCAAATGGCGTAGGCGTAGCGT
 GGGAGGTATATAAAGCAGACTCGTTAGTGAACCGTCAAGTCCGCTGGAGAC
 GCCATCCAGCTGTTTGACCTCCATAGAAGCACCGGAGCGATCCAGCTCC
 CGGGCCGGAAACGGTGCATTGGAAACCGGGATTCCCGGTGCAAAGTGA
 CCGTCGGATCTCAGCAAGCAGGTATGTAECTCTCAGGGTGGCCTGGCTTCC
 CAGTCAAGACTCAGGGATTGAGGAGCAGCTGTGGCTCTCTACATGTACC
 TTTGCTTGCCTTACACCTGACTATCTCCAGGTCAAGGATCCCAGAGTCAGGG
 CTGTTATTCTGCTGGTGGCTCCAGTTGAGAACAGTAACCCCTGCTCC
 TTGCTCTCACATCTGTCATCTCGCGAGGACTGGGACCTGTGACAAACAT
 GCTGAGTATTGGGAGGCTGGAGTGTGCGAGAGGATTCCACCTGGCAGGT
 GCTTGTGGCCTACAGTGTGGCAGTGTGAGTGTGAGTGTGAGTGTG
 GTGGGTCTCACAGTGTGGCAGTGTGAGTGTGAGTGTGAGTGTG
 TCGGACAGCTTGTGTTACATCTGAGACACAGGCCAGGTATTGAGTGTG
 AGCTTCCCACACCGCTCACGATATGAGCCTCTGAGAATTCGATTCTCAGGC
 CAGGTGATGACTCCAGGACACCTCATGCTGCTCCGCTGTGAGGCTGCG
 AGCTCAGGGATCTGTAAGGTCTATGGGACCTGGCAGTGTGAGTGTG
 GGACCACTGCTAGCCTCAGGTGGGAGCATTGAAACAGAGGAGTTG
 CCCAAGAAAATTCACTGTTGAGCTTGTGAGTGTGAGTGTG
 GCAAGTCACTTCAGGAGTCAAGGAGTCAAGGAGTCTGAGTGTGAGCTGG
 AGGGGGAAAAGCAGCTGGGAGTGTGAGTGTGAGTGTGAGTGTG
 TGTCCTCAAGGTATCACGTCATGGGAGTGAACCATGTGCTG
 GCCTTCCCTGTCACACCAAGGTGGTCAATTGGGAGTGGATCAAGGACACC
 GTGCAACCCGGGAGTGGGAGCTGGGAGCTGGGAGCTGGCAGG
 GATGTGGAAAGCAACCCAGGCCAATGGCAAGCGCGGCCCGCGCTGG
 GTGCGCTGGGGCCTGGTGTGGGGTGGCTCTCTCTCGGCTTCTCT
 CGGGTGGTTATAAAATCTCAATGAAGCTACTAACATTACTCAAAGATAATA
 TGAAAGCATTTGGATGAATGAAAGCAGAACATCAAGAAGTCTTATATAATT
 TTACACAGTACACATTAGCAGGAACAGAACAAAATTTCAGTTGAGCTGG
 ATTCATCCACGGTAAAGAATTGCGCTTGTGAGTGTGAGTGTG
 TGTCCTGTTGCTTACCCAATAAGACTCATCCAACTACATCAATAATTATGA
 AGATGAAATGAGATTTCACACATCATTATTGAAACCACTCTCCAGGATATG
 AAAATGTTGGATATTGTCACCCCTTCACTGCTTCTCTCTCAAGGAATGGCA
 GAGGGCGATCTAGTGTGAGTGTGAGTGTGAGTGTGAGTGTG
 ACGGGACATGAAAATCAATTGCTCTGGGAAATTGTAATTGCGAGATATGG
 GTTTGAGGAAATAAGGTTAAAATGCCCAGCTGGCAGGGGCAAAGGAGTCA
 TTCTCTACTCCGACCCCTGCTGACTACTTGTCTCTGGGGTGAAGTCTATCC
 TGTTGGAATTCTCTGGAGGGTGTGCAAGCTGGAAATATCCTAAATCTGA
 GGTGCAAGGAGACCTCTCACACCAAGGTACCCAGCAAATGAATATGCTTATAG
 GTGGAATTGCAAGAGGCTGGTGTCTCCAACTACATCTGTTGAGTGT
 TATGATGACAGCAAGGCTCTAGAAAATGGTGGCTCAGCACCAACAGATAG
 GCTGGAGAGGAAATCTCAAAGTGGCCTACAACTGGGACCTGGCTTACTGG
 CTTTCACACAAAAGTCAGATGACATCCACTTACCAATGAAGTGACAGAG
 TTACACATGTGATAGGTACTCTCAGAGGAGCAGTGGAAACAGACAGATATG
 TCTGGAGGTACCGGGACTCATGGTGTGGTATTGACCCCTCAGAGTGG
 AGCAGCTGGTGTCAAGAAATTGTGAGGAGCTTGGAAACACTGAAAAGGA
 TGGAGACCTAGAAGAACAAATTGTTGGCAAGCTGGGATGCAAGAAGATTG
 TTCTTGTTCTACTGAGTGGCAGAGGAGATCTCAAGACTCTCAAGACGG
 CGTGGCTTATATAATGCTGACTCATCTAGAAGGAAACTACACTCTGAGAG
 ATGTCACCGCTGATGTCAGCTTGGTACACAACTTAACAAAAGAGCTAAAG
 CCTGATGAGGCTTGAAGGAAATCTCTTATGAAAGTGGACTAAAAAGTC
 CTCCCCAGAGTCAGTGGCATGCCAGGATAAGCAAATTGGGATCTG
 TTTGAGGTGTTCTCCAACGACTTGGAAATTGCTCAGGCAGACGGTATACTA

- continued

RAW SEQUENCE LISTING

AAAATTGGGAAACAAACAAATTCA CGCGCTATCCACTGTATCACAGTGTATGAA
 ACATATGAGTTGGTGGAAAAGTTTATGATCCAATGTTAAATATCACCTCACTGT
 GC CCAAGGTTGAGGGATGGTGTGAGCTGGCAATTCCATAGTGCTCCC
 TTTGATTGTCAGGATATTGCTGAGTTAAGAAAGTATGCTGACAAAATCTACAG
 TATTCTATGAACATCACAGGAAATGAAGACATACAGTGTATCATTGATTC
 TTTTCTGCAGTAAAGAATTACAGAATTGCTTCAAGTCAAGTGAGAGACTCC
 AGGACTTGACAACAAACCAATAGTATTAGAAATGATGAATGATCAACTCATG
 TTCTGGAAAGGCAATTATTGATCCATTAGGGTTACAGACAGGCCCTTTATAG
 GCATGTATCATGTC CAAGCAGCCACAAAGTATGCAAGGGAGTCATCCCA
 GGAATTATGATGCTCTGGATATTGAAAGCAAAGTGGACCCCTTCAAGGCC
 GGGAGAAGTGAAGAGACAGATTATGTCAGCCTTCACAGTGCAGGCAGCTGC
 AGAGACTTTGAGTGAAGTACGCCAAAGATCTGGCCTAACAAAACAAAAGATG
 GGTTATTCCCTAACTTCATGGGTTACGTAATTGGAAGTTGGGGACATTGCCA
 CAAGATCATATTGACAAAAGACTGTTTAGAAACACTTCTGTAAACAG
 GCCTATTGATGAAAGTATCTCAAAAGGATTGGGGCTTTGGGCTTGCTGCTC
 CATTACACAACTGGATATTGCTTATGCTTGTATGCAATGATAACAGCT
 AACAGGCTTCACTTCTGCCAATTACAGGCTTCTAAGTAAACAGTACAT
 GAACCTTACCCCGTGC CGCAACGCCGCTGTGCGCAAGTGGTGTGAC
 GCACCCCACTGGCTGGCCATAGGCCATCGCCATCGGTGGAAACC
 TTGTTGGCTCTCGCGATCATCTGGGAAACTCCTAGCCGTTTGTGCTC
 GCAGCCGCTGGAGCAAAGCTCATAGGAATGCAATTCTGCGCTCTCGC
 GGAATATACATGTTGATCACGTATGATCTTTCCCTGCAAAATTATG
 GGACATCATGAAGCCCTTGAGCATCTGACTTCTGCTAATAAAGGAAATTATT
 TTCAATTGCAATATTGTTGGGATTTTGTCTCTCACTCGGAAGGAAATTCTGC
 ATTAATGAATCGCCAACGCCGGGGAGAGCGGTTTGCCTATTGGGCTCTT
 CCGCTTCTCGCTCACTGACTCGCTCGCCTCGTGTGGCTGCGGAGCGG
 TATCAGCTCACTCAAAGCGGTAAATACGGTTATCCACAGAATCAGGGATAACGC
 AGGAAAGAACATGTGAGCAGGAAAGGCCAGGAACCGTAAAAGGC
 CGCGTTCTGGGTTTCCATAGGCTCGCCCCCTGACGAGCATCACAAAT
 CGACGCTCAAGTCAAGGGTGGCAAAACCGACAGGACTATAAAGATACAGGCG
 TTCCCTGAGGAGCTCCCTGTCGCTCTCTGTTCCGACCCCTGCCGTTACCG
 GATACTGTCGCCCTTCTCCCTCGGAGCTGGCGCTTCTCATGCTCAG
 CTGTTAGGATATCTGAGTTCGGTGTAGGTCGCTCCAAGCTGGCTGTGAC
 GAACCCCCGTTAGCCGACCGCTGCCATTACCGTAACTATCGTCTGAGT
 CCACCCGGTAAGACAGCACTATGCCACTGGCAGCAGGACTCGTAACAGGA
 TAGCAGAGCGAGGTATGAGGCTGTCAGAGTTCTGAGAAGTGGTGGCCTA
 ACTACGGCTCAACTAGAAGAACAGTATTGGTATCTGCGCTCTGTAAGCCAGT
 TACCTTGGAAAAGAGTTGGTAGCTTGATCCGCAAACAAACACCCTGGT
 AGCGGTGTTTTTGTGCAAGCAGCAGATACCGCAGAAAAGGATCTC
 AAGAAGATCTCTGATTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAATC
 ACCTTAAGGGATTGGTGTAGGATTATCAAAGGATCTCACCTAGATCCTT
 TAAATAAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGCTG
 ACAGTACCAATGCTTAATCAGTGAGGACCTATCTAGCGATCTGTATTCG
 TCATCCATAGTTCGCTGACTC

SEQ ID NO: 30. NUCLEOTIDE SEQUENCE OF PLASMID 449
 GCGTAATGCTGCCAGTGTACAACCAATTAAACCAATTCTGATTAGAAAAC
 ATCGAGCATCAAATGCAACTGCAATTATTGATCATGAGATTATCAATACCCATT
 TTGAAAAGCGTTCTGTAATGAAAGGAAAAGTACCCGAGGCTTCCATAGG
 ATGGCAAGATCTGGTATCGCTCGGATTCCGACTCGTCAACATCAAC
 CTATTAACTCCCTCGTCAAAATAAGGTTATCAAGTGAAGAATCACCAGT
 AGGACTGAACTGGTGAAGATGGCAAAGGCTATCGTCAAGTGGACTTGT
 AACAGGCGAGCCATTACGCTCGTCAAAATCACTCGCATCAACCAACCGTT
 TTCATTGCTGATTGCCCTGAGCGAGACAAATCCGATCGCTTAAAGGAC
 ATTACAAACAGGAATCAATGCAACCGGCGAGGAACACTGCCAGCGCATCAAC
 ATAATTCTCCTGTAATCAGGATATTCTCTAATACCTGGAATGCTGTTCCCG
 GGATCGCAGTGGAGTAACCTGCACTCAGGAGTACGGATAAAATGCTTGAT
 GCTGGAAGAGGCTAAATTCCGTCAGCCAGTTAGTCTGACCATCTCATCTGTA
 ACATCATTGGCAACGCTACCTTGCATGTTCAGAAACAACTCTGGCGATCGG
 GCTTCCCATACATCGTATGCTGCGACCTGATGCCCCGACATTATCGCGAGC
 CCATTATACCCATATAAATCAGCAGTATGGGATTTAATCGGCGCTCGAGC
 AGAGCTTCCGTTGAATATGGCTCATACACCCCTTGATTAATCTGTTATGTA
 GCAGACAGGTGACAATATTGGTATGGGATTGGCATACGTTGATCTATATCAT
 ATATGTAACATTATATTGGCTCATGTCATATGACCGCCATGTTGACATTGATTA
 TTGACTAGTTAATAGTAATCAATTACGGGCTTACGGGATTTCTACGGCCATATATG
 GAGTTCCGCTTACATAACTTACGGTAAATGGCCCGCTGGCTGACGCCAAC
 GACCCCGCCATTGACGTAAATAATGACGTATGTTCCCATAGTAACGCCAATAG
 GGACTTCCATTGACGTCAATGGGAGTATTACGGTAAACTGCCACTTGGC
 ATGACATCAAGTGTATCATGCAAGTCCGCCCTATTGACGTCAATGACGGT
 AAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTCTACTT
 GGCAGTACATCTACGTATTAGTCACTCGCTTACCATGGTATGCGGTTTGGCA
 GTACACCAATGGCGTGGATAGCGGTTTGACTCACGGGATTTCAGTCTCCAC
 CCCATTGACGTCAATGGGAGTTGTTGGCAGGAAATCAACGGGACTTCCAA
 ATGTCGTAATAACCCGCCCGTTGACGCAAATGGCGTAGGCGTGTACGGT
 GGGAGGTCTATAAGCAGAGCTCGTTAGTGAACCGTCAGATGCCCTGGAGAC
 GGCATCCACGCTGTTGACCTCATAGAAGACACCGGACCGATCCAGCCTCC
 CGGGCGGAAACGGTGCATTGAAACCGGATTCCCGTGCAGAGTGA
 CTCA

- continued

RAW SEQUENCE LISTING

CGTCCGGATCTCAGCAAGCAGGTATGACTCTCAGGGTGGGCCTGGCTTC
 CAGTCAAGACTCAGGGATTGAGGGACGCTGGCTCTCTTACATGTACC
 TTTGCTTGCTCAACCTGACTATCTCCAGGTAGGATCCAGAGTCAGGGT
 CTGATTTCTCGTGGCTCAGTTCAAGAACAGTAACCCCTGCTCCGAATA
 TTGCCCTCTCACATCTCGTCACTCTCCGGAGGACTGGGACCTGTGACAAACAT
 GGCTAGCGCGCCGCCGCGCTGGCTGTGCGCTGGGCGCTGGTGTGGCG
 GTGGCTCTCTCTCGGCTCTCGGGTGGTTATAAAAATCCTCCAAATGA
 AGCTACTAACATTAACCAAGCATATAATGAAAGCATTTGGATGAATTGAAAG
 CTGAGAACATCAAGAAGTTATAATTACAGATACCAATTAGCAGGA
 ACAGAACAAAATTCAGCTGCAAGCAAATTCAATCCCAGTGGAAAGAATTGG
 CCTGGATTCTGTGAGCTGGCACATTATGATGTCTGTGCTCACCAAAATAAGA
 CTCATCCCAAATCACATCTAACATTAAATGAAGATGAAATGAGATTTCACACAT
 CATTATTGAAACCACTCCCTCAGGATATGAAATTTCCGATATTGACCAAC
 TTCAGTGTCTCTCTCAAGGAATGCCAGAGGGCGATCTAGTGTATGTTA
 ACTGCAAGACTGAAGACTTCTTAAATTGGAACGGGACATGAAAATCAATTGCTCG
 GGAAAATTGTAATTGCAAGATATGGAAGTTTCAAGAGGAATAAGGTTAAAAT
 GCCCAGCTGGCAGGGCCAAGGAGTCATTCTACTCCGACCCCTGCTGACTAC
 TTGCTCTGGGTGAAGTCTATCCAGATGGTTGAATCTTCTGGAGGTGGT
 TCCAGCGTGGAAATCTCTAAATCTGAATGTTGAGGACCCCTCACAACCAAG
 TTACCCAGCAAAATGATGTTATAGGCTGGAATTGCAAGGGCTGTTGCTT
 CCAAGTATTCTGTTATCCAAATTGGAATCTATGATGCACAGAACGCTCTAGAAAA
 AATGGGTGGCTCAGCACCAAGATAGCAGCTGGAGAGGAAGTCTCAAAGTGC
 CTACAACTGTTGGACCTGGCTTACTGGAAACTTTTACACAAAAGTCAAGATGC
 ACATCCACTCTACCAAAATGAAAGTGAAGAATTAACATGTGATAGGTACTCTCAG
 GGAGCAAGTGGAAACAGACAGATATGTCATTCTGGAGGTACCGGACTCATGG
 GTGTTGGTGTATTGACCTCAGTGGAGCAGTGTGTTCATGAAATTGTA
 GGAGCTTGGAAACACTGAAAAGGAAGGGTGGAGACCTAGAAGAACAAATTGTT
 TCAAGCTGGGATGCAAGAAGATTGGCTCTTCTGGTTACTGAGTGGCAGAG
 GAGAATTCAAGACTCTTCAAGAGCTGGCTGGCTTATTAATGCTGACTCAT
 CTATAGAAGGAAACTACACTGAGAGTTGATGTACACCGCTGTGACAGCTT
 GTACACAAACCTAACAAAGAGCTGAAAAGCCCTGATGAAAGCCTTGAAGGCCAA
 TCTCTTATGAAAGTCTGCTTCCAGGAAATTGCAAGGTTCTGGCATGCC
 CAGGATAAGCAATTGGGATCTGGAAATGATTGAGGTGTTCTTCAACGACTTG
 GAATTGCTTCAGGCAGAGCAGGTATACTAAAATGGGAAACAAACAAATTG
 CGGCTTCAACTGATCACAGTGTATGAAACATATGAGTTGGTGAAAAGTTT
 ATGATCAAATGTTAAATATCACCTCACTGTGAGGCTTCAAGGAGGATGGT
 GTTGAGCTGGCAATTCCATAGTGTCTCCCTTTGATTGTCGAGATTATGTTG
 TTTAAGAAAGTATGTCGACAAATCTACAGTATTCTATGAAACATCACACAGGAA
 TGAAAGACATAAGTGTATCATTTGACTTTCTGAGTAAAGAATTTCAG
 AAATTGCTTCAAGTCTAGTGGAGAGACTCCAGGACTTTGACAAAAGCAACCCAAATA
 GTTAAAGAATGATGATCAACTCATGTTCTGAAAGAGCATTTATGATCC
 ATTAGGGTTACAGACAGGCTTTTATAGGCATGTCATCTATGCTCAAGCAGC
 CACAACAAGTATGCAAGGGACTCATCCCAAGGAAATTATGATGCTCTGTTGATAT
 TGAAGAACAGTGGACCTTCAGGCTGGGAGAAGTGAAGAGACAGATTAT
 GTTGAGCTCTCAAGCTGAGCTGGAGAGACTTTGAGTGAAGTAGCTAA
 GATCTGACCCCCCTAACGTTACTGGCGAAGCGCTTGGAAATAAGCCGGTGTG
 GTTGTCTATGTTATTTCACCATATTGCGTCTTGGCAATGTGAGGGCCC
 GGAAACCTGGGCTCTCTGAGGAGCATTCAGGGGCTTTCAGGGGCTTCCCTCGC
 CAAAGGAATGCAAGGCTGTTGAATGTCGTGAAGGAAGGACTCTCTGAAAGCT
 TCTTGAAGACAAACACGTCCTGAGCGACCTTGCAGGACGGAAACCCCCCA
 CCTGGCGACAGGGCTCTCGGCCAAAGCCACGTGTATAAGATAACCTGCA
 AAGGCGCACAGGCTCCAGTGGGAGACTGCAAGGAGGACTTGTGAGGAAAGGTC
 AAATGGCTCTTCAAGCGTATTCACAAAGGGCTGAAGGGATGCCAGAGGTAC
 CCCATTGTTAGGGATCTGATCTGGGCTCGGTGACATGTTTACATGTT
 GTGAGGTTAAAAACGCTAGGCCCGAACACGGGACGTGGTTTCTT
 GAAAAACACGATGATAATATGCCAGCAAGGGCTGTGCTGCTGGCTGTGATGG
 CAGGCTGGCCCTGAGGCCAGGACTGCCCCTGCTGTGCTACTCTGCAAGGCC
 AGGTGAGCAACGAGACTGCCAGTGGAGAACCTGCAACCCAGCTGGGGAG
 CAGTGTGGACCGCGCATCCGCAGTGGCTCTGACCGTACAGCAA
 GCCTGAGCTGAACTGCGTGGATGACTCACAGGACTACTACGTTGGCAAGAAG
 AACATCACGTGCTGAGCACCGACTTGTGCAACGCCAGCGGGGCCATGCCCTG
 CAGGCCGGCTGCCATCTGCGCTGCTCCCTGACTCGGCTGTGCTCTGG
 GGACCCGGCCAGCTAGGGATCTGGGCCATAACAAAACAAAAGATGGGTTA
 TCCCCTAAACTCATGGTTAGTAATTGAAAGTTGGGACATTGCCACAAGAT
 CATATTGTAACAAAGTCAAACACTGTTTAAAGAAAATTCTGTAAACAGGCTATT
 GATTGGAAAGATGTCAAAGGATTGTTGCTTGGCTTGCTGCTCCATTAC
 ACAATGTTGATATCTGCCCTTATGCCCTTGTGATGATACAGCTAACACAGG
 CTTTCACTTCTGCCAACTTACAAGGCCCTTCTAAGTAAACAGTACATGAAACCTT
 ACCCCGTTGCTGGCAACGGCTGGCTGTGCAAGTGTGTTGCTGACGCAACCC
 CCACTGGCTGGGCTGGCATAGGCCATAGCGCAGTGGCTGGAACCTTGTG
 CTCCCTGCCGATCCACTGCGGAACTCCCTAGCGCTTGTGCTGCGAGCCG
 GCTGGAGCAAGCTCATAGGAACGACAAATTCTGCTGTCTCCCGGAAATAT
 ACATCGTTGATCTACGTTGATCTTCTGGCTGTGCAAAATTATGGGACAT
 CATGAAGCCCCCTGACCATCTGACTCTGGTAAATAAGGAAATTATTCATTG
 CAATAGTGTGTTGAAATTGGTGTCTCTCACTCGGAAGGAATTCTGATTAAATG
 ATCGCCCAACGCCGGGGAGAGGCGGTTGCGTATTGGGCGCTTCCGCTTC
 CTCGCTCACTGACTCGCTGCGCTCGTGGCTGCGGAGCGGTATCAGC

- continued

RAW SEQUENCE LISTING

TCACTCAAAGGCGGTAAACGGTTATCCACAGAATCAGGGATAACGCAGGAAAG
 AACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGCGTG
 CTGGCGTTTCTCATAGGCTCGGCCCTGACGCCAGACATCACAAAATCGACGCT
 CAAGTCAGGGTGGCGAAACCCGACAGGACTATAAAGATAACCGAGCTTCCCC
 CTGGAAGCTCCCTCGTGCCTCTCTGTTGACCCCTGCCCTAACGGATAACCT
 GTCCGCCTTCTCCCTCGGAAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGG
 TATCTAGTTGGTAGGTCGGCTCCAGGCTGGGCTGGTGCACGAAACCC
 CGCTTACGGCCGACCGCTGCCCTTACCGTAACATCGTCTGAGTCCAACCC
 GTAAAGACACGACTATCGCCTACTGCCAGCAGGACTGGTAACAGGATTAGCAG
 AGCAGGATATGAGCGTCTACAGAGTTCTGAAAGTGGTGGCCTAACTACGG
 CTACATAGAAACAGTATTGGTATCTGCTCTGCTGAAGCAGTTACCTTC
 GAAAAAGAGTTGGTAGCTTGTACGCCCCAAACAAACCCCGCTGGTACCGGT
 GGTGTTTTGTTGCAAGCAGATTACGCCAGAAAAAAAGGATCTAAGAAG
 ATCCTTGATCTTCTACGGGCTGACGCTCAGTGGAAACGAAACTCACGTTAA
 GGGATTGGTACGAGATTCAAAAGGATCTTCACCTAGATCTTTAAATTAA
 AAATGAAGTTAAATCAATCTAAAGTATATGAGTAACCTGGTCTGACAGTTAC
 CAATGCTTAATCAGTGGGACCTATCAGCAGATGTCTATTGTTGTTCATCCAT
 AGTTGCCTGACTC

SEQ ID NO: 31. NUCLEOTIDE SEQUENCE OF PLASMID 603
 GGCGTAATGCTGCCAGTGTACAACCAATTACCAATTCTGATTAGAAAAACTC
 ATCGAGCATCAAATGAAACTGCAATTATTCATATCAGGATTATCAATTACCATATTT
 TTGAAAAGGCCCTTCTGTAATGAAAGGAAAACCTACCCGAGGCACTTCCATAGG
 ATGGCAAGATCTGGTATCGGCTCGGATTCGACTCGTCCACATCAATACAC
 CTATAATTCCCTCGTCAAAAATAAGGTATCAAGTGAAGAAATCACCATGAGTG
 ACGACTGAATCCGGTGAGAATGGCAAAGCTTATGCAATTCTTCAGACTTGTG
 AACAGGCGAGGCTTACGCTGTCATCAAATCACTCGCATCAACCCAAACCGTTA
 TTCACTTGTGATGCCCTGAGCGAGAACATCGCGATCGCTTAAAGGAC
 ATTACAAACAGGAATCAAATGCAACCGGGCGAGGAACACTGCCAGCGATCAAC
 ATATTTCACTGAACTAGGATATTCTCTAATACCTGGAATGCTGTTTCCGG
 GGATCGCAGTGTGAGTAACCGTACATCAGGAGTACGGGATAAAATGCTTGAT
 GGTGGAAGAGGCTATAATTCCGTAGCCAGTTAGCTGACCATCTCATCTGTA
 ACATATTGGCAACGCTACCTTGTGATGTTGCAAAACACTCTGCGCATCGG
 GCTTCCATACATCGATAGATTGTCGACCTGATTGCCGACATTATCGCGAGC
 CCATTATACCCATATAATCAGCATCATGTGAAATTAAATCGGGCCTCGAGC
 AGAGCAGTCCCCTGTAATATGGCTCATACACCCCTTGATTAATCTGTTATGTA
 GAGACAGGTCGACAATATTGCTATTGGCATTGCACTACGTGATCTATCAT
 AATATGTACATTATATTGGCTCATGTCATATGACCGCCATTGACATTGATTA
 TTGACTAGTTAAATAGTAATCAATTACGGGCTATTAGTTCATAGCCATATAG
 GAGTTCCCGCTTACACTACGGTAAATGGCCCGCTGGCTGACCGGCCAAC
 GACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
 GGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAACCTGCCACTTGGC
 AGTACATCAAGTGTATCATGCCAAGTCCGCCCTATTGACGTCATGACGGT
 AAATGGCCCGCTGGTATTGCCCCAGTACATGACCTTACGGGACTTCTACTT
 GGCAGTACATCTACGTATTAGTCATGCTTACCATGGTATGCGGTTTGGCA
 GTACACCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCAC
 CCCATTGACGTCAATGGGAGTTGTTGGCACCAAAATCACGGGACTTCCAA
 AAATGCTTAATTAAGCAGACTCGTTAGTGAACCGTCAGATCGCTGGAGAC
 GGGAGGCTATAAAGCAGACTCGTTAGTGAACCGTCAGATCGCTGGAGAC
 GCCATCCACGCTTTGACCTCATAGAACAGCACGGGACCGATCAGCTCC
 GCGGCCGGAACGGTCATTGGAACGCCGATTCCCGTGCCAGAGTGAAC
 CGGCGGATCTCAGCAAGCAGGTTGACTCTCCAGGGCTGGCTGGCTTCCC
 CAGTCAAGACTCAGGGATTGAGGATCTCAGGAGCTGGCTGGGCTTCTTACATGACC
 TTTGCTTGCCTCAACCTGACTATCTCCAGGTCAAGGATCCAGAGTCAGGGT
 CTGTATTTCCTGCTGGCTCCAGTCAGGAACAGTAAACCCCTGTCGGATA
 TTGCTTCTCATACTCGTCAATCTCCGGAGACTGGGACCCCTGTGACGAACAT
 GGCTAGCAAGGTGTGCTGGCTTGTGATGGCAGGGCTGGCCCTGAGCC
 AGGCACCTGCCCTGCTGTCTACCTGCAAAAGCCAGGTGAGCAACGAGGACTG
 CCTGCAGGTGGAGAACTGCACCCAGTCGGGGAGCAGTGTGGACCGCGC
 TCCGGCAGTGGCCCTCGACCTGACCGTCATCACGAAAGCTGAGCTTGAAC
 TGGATGACTCACAGGACTACTACGGTGGCAAGAAGAACATCACGTGCTGTGACAC
 CGACTTGTGCAACGCCAGCGGGCCCATGCCCTGAGCCGGCTGCCGACATCC
 TTGCGCTGCTCCCTGCACCTGCCCTGCTGCTGGGACCCGGCAGTATA
 GATCTGACCCCTAACTGTTACTGGCGAAGCCGCTTGGAAATAAGGCCGTG
 GTTTGCTTATGTTATTTCACCATATTGCGTCTTGGCAATGTGAGGGGCC
 GGAACCGCTGGCCCTGCTTCTGACGAGCATTCTGAGGGCTTCCCTCTCGC
 CAAAGGAATGCAAGGCTGTTGAATGTCGTGAAGGAAGCACTCTCTGAAAGCT
 TCTTGAAGACAACAAACGTCGTTAGCGACCTTGGCAGGAGCGGGAAACCCCA
 CCTGGCGACAGGTGCTCTGCCAAAAGCCACGTGTATAAGATAACACCTGCA
 AAGGCGCACAACCCAGTGCACCGTGTGAGTTGGATAGTTGTGAAAGAGTC
 AAATGGCTCTCTCAAGCGTATTCACAAAGGGCTGAAGGGATGCCAGAGGTAC
 CCCATTGATGGGATCTGATCTGGGCTCGGTGACATGCTTACATGTTTA
 GTCGAGGTTAAAAACGCTAGGCCCCCGAACACGGGGACGTGGTTTCTCTT
 GAAAAACACGATGATAATATGCCACAACCATGGCGGCCCGGGCTGGCTG
 TCGCTGGGGCGCTGGCTGGCGGGCTTCTTCTCTGGCTTCTCTC
 GGGTGGTTATAAAATCTCCATGAAGCTACTAACATTACTCCAAAGCATATA
 GAAGCATTTGGATGAATTGAAAGCTGAGAACATCAAGAAGTTCTTATATAATT

- continued

RAW SEQUENCE LISTING

TACACAGATAACCACATTAGCAGGAACAGAACAAAACCTTCAGCTGCAAAGCAAA
 TTCATCCAGTGGAAAAGAATTGGCTGGATTCTGTTGAGCTGGCACATTATGAT
 GTCCCTGTGCTTACCCAAAATAGACTCATCCAACACTACATCTAATAATTATGAA
 AGATGGAAATGAGATTTCAACACATCATTATTAAGGACACCTCCAGGATATG
 AAAATTTCCGATATTGACCACTTCAGTGCTTCTCTCCAAGGAATGCCA
 GAGGGCGATCTAGTGTATGTTAATGCACGAACATGAAGACTCTTAAATTGGA
 ACGGGCATGAAATCAATTGCTCTGGAAAATTGTAATTGCGAGATATGGAAA
 GTTTCAGGAAAATAGGTTAAAATGCCAGCTGGCAGGGGCAAAGGAGTCA
 TTCTACTCCGACCTGCTGACTACTTGCTCTGGGTGAAGTCTATCCAGA
 TGTTGGAATCTCCTGGAGGTGGTCCAGCGTGGAAATATCCTAAATCTGAAT
 GTGAGGAGACCTTCACACCCAGGTTACCCAGAAATGAATATGCTTATAGGC
 GTGAAATTCCAGAGGCTTCTTCAGTATTCTGTCACTCAATTGGATAC
 TATGATGCACAGAAGCTCTAGAAAAAATGGTGGCTCAGCACCAACAGATAGCA
 GCTGGAGAGGAAGTCTCAAAGTGCCTACAATGTTGGACCTGGCTTACTGGAA
 CTTTCTACACAAAAGTCAAGATGCACATCCTACCAATGAAGTGAACAGAA
 TTACATGTTAGGTACTCTCAGAGGAGCAGTGGAAACAGACAGATATGTCAT
 TCTGGAGGTACCGGGACTATGGTGTGTTGCTTACAGGCTCAGAGTGG
 AGCAGCTGTTGTCATGAAATTGAGGAGCTTGGAACACTGAAAAAGGAAGGG
 TGGAGACTGAAAGAACAAATTGTTGCAAGCTGGGATGCGAGAAGAATTGGTC
 TTCTGGTTCTACTGAGTGGCAGAGGAAATTCAGACTCCTTCAGAGCGTGG
 CGTGGCTTATAATTAGCTACTCATATAGAAGGAACACTACTCTGAGAGTTG
 ATTGTACACCGCTGATGTCAGCTGGTACACAACTAACAAAAGAGCTGAAAAG
 CCTGATGAAAGCTTGAAGGCAAATCTCTTATGAAAGTGGACTAAAAAAAGTC
 CTTCCCAGAGTCAGTGGCATGCCAGGATAAGCAAATTGGGATCTGGAAATG
 TTTGAGGTGTTCTCCAAAGACTTGGAAATTGCTTCAAGGAGACCTTCAGAGCGTGG
 AAAATTGGGAAACAAACAAATTAGCGGGCTTACACTGTTACAGTGTCTATGAA
 ACATATGAGTTGGTGGAAAATTGATCCAAATGTTAAATATCACCTCACTGT
 GGCCAGGTTGGAGGGGATGGTGTGAGCTGGCAATTCCATAGTGTCTCC
 TTTGATTGTCAGATTATGCTGTTAGTTAGAAGAAGTATGCTGACAAAATCTACAG
 TATTCTATGAAACATCCACAGGAATGAAGACATACAGTGTATCTTGTACT
 TTTTCTGCTGAAAGAATTTCAGAAATTGCTTCAAGGTTCAAGTGGAGAGACTCC
 AGGACTTTGACAAAGCAACCCAAATAGTATTAGAATGATGATGTCACACTCATG
 TTCTGGAAAGAGCATTATTGATCATTAGGGTTACCGACAGGCTTTTATAG
 GCATGTCATCTGTCAGCAGGCCAACAACTGTTAGAAAACCTTCTGTAACAG
 GGAATTATGATGCTCTGTTGATATTGAAAGCAGGAAATTGGACCCCTCCAGGCCTG
 GGGAGAAGTGAAGAGACAGATTATGTTGCAAGCTTCAAGTGGCAGCAGCTGC
 AGAGACTTGAAGTAGCTAAAGATCTGGGCTTAACAAAACAAAAGATG
 GGGTTATTCCCTAACTTCTAGGGTTACGTAATTGGAAGTTGGGGACATTGCCA
 CAAGATCATATTGAAACAGTCAACACTGTTTAGAAAACCTTCTGTAACAG
 GCCTATTGATGAAAGTATGTCAGGAAATTGGGCTTTGGCTTGTGCT
 GGGAGAAGTGAAGAGACAGATTATGTTGCAAGCTTCAAGTGGCAGCAGCTGC
 CATTACACATGTTGATCTGCTTATGCTTGTGATGCACTGATAACAGCT
 AACACGGCTTCACTTCTCGCAACTTACAAGGCTTCTAAGTAAACAGTACAT
 GAACCTTTACCCGTTGCTCGCAACCGCCCTGTCAGTGGCAAGTGTGTC
 GCACCCCCACTGGCTGGGGCTGGCCATAGGCCATCAGGCCATCGTGGAAACC
 TTGTTGGCTCTCGCGATCCACTCGCGAACCTCTAGCGCTTGTGCT
 CGAGCCGCTTGGAGAAAGCTCATAGGAACTGCAATTCTGCTGCTCTCGC
 GGAATATACATGTTGATCAGTATGATCTTCCCTGCAAAATTATG
 GGACATCATGTTGAGGCTTGGCATCTGACTCTGCTAATTAAAGGAAATTCT
 TTGATTGCAATAGTGTGTTGAAATTGTTGTCCTCACTCGGAAGGAAATTCTG
 ATTAATGAATCGCCAAACGCCGGGGAGAGGGGTTGCGTATTGGCGCTCTT
 CCGCTTCCCTCGCTCACTGACTCGCTCGCCTCGCTCGCTCGGGCGAGCG
 TATCAGCTCACTCAAAGCGCTAATACGGTTATCCACAGAATCAGGGATAACGC
 AGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAGGC
 CGCGTTGCTGGCTTTCCATAGGCTCCGCCCTGACGGCATCACAAAAT
 CGACGCTCAAGTCAAGGGTGGCAAACCCGACAGGACTATAAAGATACAGCG
 TTGCGCTTGGAGGCTCCCTCGCTCGCTCTCTGTTCCGACCCCTGCCGTTACCG
 GATACCTGTCGCCCTTCCCTCGGGAGCGCTTGTGCTCGCTCGGGCTTCTCATAGCTCACG
 CTGTTAGGTATCTAGTTGGTGTAGGCTGCTCGCTCAAGCTGGCTGTGAC
 GAACCCCCGTTAGCCGACCGCTGCCATTACCGTAACATCGTCTGAGT
 CCACCCGGTAAGACAGACTATCGCCACTGGCACGCCACTGGTAACAGGA
 TTAGCAGAGCGAGGTATGAGGGCTGACAGGTTCTGAGGTGGCT
 ACTACGGCTCACTAGAAGAACAGTATTGTTGATCTGCGCTCTGCTGAAAGCG
 TACCTCGGAAAAGAGTTGGTAGCTTGATCCGCAAACAAACACCAGCTGGT
 AGCGGTTGTTTTTGTGCAAGCAGCATACCGCAGAAAAGGATCTC
 AAGAAGATCTCTGATCTTCTACGGGCTGACGCTCAGTGGAAACGAAAAC
 ACGTTAGGGATTGGTGTAGGATATTCAAAAGGATCTCACCTAGATCCTT
 TAATTAATTAATGAAGTTAAATCAATCTAAGTATATGAGTAAACTTGGCT
 ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTG
 TCATCCATAGTGTGCTGACTC

SEQ ID NO: 32. NUCLEOTIDE SEQUENCE OF PLASMID 455
 GCGTAAATGCTGCCAGTGTACAACCAATTGATAGAAAAACTC
 ATCGAGCATCAAATGAAACTGCAATTATTATCATATCAGGATTATCAATTACCATATT
 TTGAAAAGCGCTTCTGTAATGAAGGAAAACCTACCGGAGGCAGTCCATAGG
 ATGGCAAGATCTGGTATCGCTCGCATTCCGACTCGTCAACATCAATTACAC
 CTATTAATTCCCTCGTCAAAATAAGGTTATCAAGTGAAGAACCATGAGTG
 ACGACTGAATCCGGTGAGAATGGCAAAGCTTATGCTTCCAGACTTGGTTC

- continued

RAW SEQUENCE LISTING

AACAGGCCAGCCATTACGCTCGTCATCAAATCACTCGCATCAACCAAACCGTTA
 TTCACTCGTATTGCGCTGAGCGAGACGAAATACCGATCGCTGTTAAAGGAC
 AATTACAAACAGGAATCAAATGCAACGGCCAGGAACACTGCCAGCGCATCAAC
 AATATTTCACCTGAATCAGGATATTCTCTATACTCTGGAATGCTGTTTCCGG
 GGATCGCAGTGACTAACCATGCATCATCAGGAGTACCGATAAAATGCTGAT
 GGTGCGGAAGAGGCCATAAATTCCGTAGCCAGTTAGTCTGACCATCTCATCTGA
 ACATCATGGCAACGCTACCTTTCAGGAAACACTCTGGCGCATCGG
 GCTTCCCATAACATCGATAGATTGCGCACCTGATTGCCGACATTATCGGAGC
 CCATTATACCCATATAAATCAGCATCCATGTGAAATTAAATCGGGCCTCGAGC
 AAGACGTTCCCGTGAATATGGCTCATAAACCCCCCTGTATTACTGTTATGTA
 GCAGACAGGTGATGGCTATTGGCCATACGTTGATCTATATCAT
 ATATATGACATTATATTGCTCATGCAATATGACGCCATGTTGACATTGATTA
 TTGACTAGTTAATAGTAATCAATTACGGGTATTAGTCATAGCCATATATG
 GAGTCGCCGTTACATAACTACGGTAAATGGCCGCCCTGGCTGACC GCCAAC
 GACCCCGCCATTGACGCTAAATAATGACGTTAGTTCCTAGTAACGCCAATAG
 GGACTTTCCATTGACGCTAATGGGTGGAGTATTACGGTAAACTGCCACTTGGC
 AGTACATCAAGTGTATCATGCCAAGTCCCGCCCTATTGACGTCATGACGGT
 AAATGGCCGCCGCTGGCATTAGGCCAGTACATGACCTTACGGGACTTTCTACTT
 GCGAGTACATCACGCTATTGACGCTAAATAATGACGTTAGTTCCTAGTAACGCCAATAG
 GTACACCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCAC
 CCCATTGACGTCATGGGAGTTGTTGGCACC AAAATCACGGGACTTTCCA
 AATGTCGTAATAACCCGCCCGTGTGCGAAATGGCGTAGGCGTAGCGT
 GGGAGGTCTATAACAGGACTCTGTTAGTGAACCGTCAGATGCCCTGGAGAC
 GGCATCACCGCTTTGACCTTCAAGAGACACC GGACGATCCAGCCTCC
 CGGGCGGGAAAGGGCATTGGAAACCGGATTCCCGTGTGCGCAAGAGTCA
 CCGTCCGGATCTCAGCAAGCAGGTATGTA CTCCAGGGTGGGCTGGCTTCCC
 CAGTCAGACTCAGGGATTGAGGGACGCTGTGGCTTCTCTTACATGTA
 TTTGCTTGCCTCAACCCCTGACTATCTCCAGGTCAAGGATCCAGAGTCAGGGT
 CTGTATTTCCTGCTGGCTCCAGTCAGAACAGTAAACCCCTGCTCCGAATA
 TTGCTCTCACATCTCGTCATCTCCCGAGGGACTGGGACCTGTGACGAACAT
 GCTGAGCAAGGCTGTGCTGCTTGTGAGTGGCAGGCTGGGCTGTGAGCC
 AGGCACTGGCTCTGTGCTACTCCTGAGGCTGGGACGGTGTGAGCAACGGAGACT
 CCTGCAGGTGGAAACTGCCACAGCTGGGGAGCAGTGCTGGACCGCGC
 TCCGCGAGTGGCCTCTGACCGTCAGCAAAGGCTGAGCTGACTGCG
 TGGATGACTCAAGGACTACTAGTGGCAAGAAGAACATCACGTGCTGTGACAC
 CGACTTGCACGCCAGCGGGGCCATGCCCTGAGCCGGCTGGCCCATCC
 TTGCGCTGCTCCCTGCACTCGGCTTGCTCTGGGACCCGGCAGCTAGA
 GATCTGACCCCCCTAACGTTACTGGCGAAGCCGTTGGAAATAAGCCGGTGTG
 GTTTGCTATATGTTATTTCCACCATATTGCGCTTTTGCAATGTGAGGGCC
 GGAAGAACCTGGCTGCTTCTGACGAGCATTCTAGGGGCTTTCCCTCTCGC
 CAAAGGAATGCAAGGCTGTGAAATGCTGTAAGGAGGACTCTCTGTGAAAGCT
 TCTTGAGAACAAACAACGTCTGTAGGGACCTTTCAGGCGAGCGGAAACCCCA
 CCTGGCGACAGGTGCTCTGGGCCAAAAGCCACGTGTATAAGATAACCTGCA
 AAGGCGCACACCCAGTGGCACCGTGTGAGTTGGATAGTTGTGAGAACAGTC
 AAATGGGCTCTCCAGTCAGTGGGCTCTGAGGATCTGAGGATGCCCAGAAGGTC
 CCCATTGATGGGATCTGATCTGGGCTCTGGCACATGCTTACATGTGTTA
 GTCGAGGTTAAAAAACGCTAGGCCCCCGAACACGGGAGCTGGAGTGCAGAAG
 CATTCCCAACCTGGCAGGTGCTTGCGCTCTCGTGGCAGGGCAGTCTGGC
 GTGTTCTGGTCACCCCACTGGGCTCTCACAGCTGCCACTGCATCAGAAC
 AAAAGCGTGTACTTGTGGTCGGCACAGCTGGTGTTCATCTGAAGACACAGGCC
 AGGTTAGTCAGGCTCAGGCCACAGCTTCCACACCCGCTACAGATATGAGCTC
 GAAGAATGATTCTCAGGCCAGGTGATGACTCCAGCCACGCCATGCTGCTC
 CGCTGTGAGGCTGCCAGCTCAGGATGCTGTGAAGGTCACTGGACCTGCC
 ACCCAGGAGGCCACTGGGACCACCTGCTACGCCCTAGGCTGGGAGCAGCAT
 TGAACCGAGGAGTTCTGACCCAAAAGAACCTTCAAGGCTGAGGCTGGGCTTCTG
 ATTCCAATGACGCTGTGCGCAAGTTCCACCTCAGAAGGTGACCAAGTTCA
 TGTTGCTGGAGGCTGGACAGGGGAAAAGCACCTGCTCGGGTATTCTGG
 GGGCCACTTGTGTAATGGTGTGCTTAAGGTATCACGTATGGGCACTGT
 CATGTGAGGTTGGGCTGGGAGCAGCATGTTGAGGTTGGGCTGGGAG
 CTTGTAACAGGCCATTGATGGGAAAGTATGTCAGGAACTGGGCTGGGCTGG
 GCTTGTGCTGCTTACACATGTGGGATATCTGCTTAATGCTTGTGTTATGCA
 TGTTACAGCTAACCTTACCCCTGCTGCCAACACTTACAAGGCTTTCTAAG
 TAAACAGTACATGAACTTACCCCTGCTGCCAACGGCTGGCTGTGCAA
 GTGTTGCTGACGCAACCCCACTGGCTGGGCTGGCCATAGGCCATGCC
 ATGCGTGGAACCTTGTGGCTCTTGCCGATCCACTGCGGAACCTCTAGCCG
 CTGTTTGCTGCGAGCGGTCTGGAGCAAGGCTACAGGAACTGACAATTCTG
 CGTCCCTCGCGGAATATACATCGTTGACTACGTTGATCTTCTGGCT
 CCAAAATATGGGGACATCATGAAGCCCTTGAGCATCTGACTCTGGCTA
 AGGAAATTATTTTATTGCAATAGTGTGTTGAAATTGGTGTCTCACTCG
 AGGAATTCTGCAATTGTAATGCAACGCCAGCGGGAGAGGCCGTTGGCT
 TGGCGCTCTCCGCTCTCGTCACTGACTCGCTGCGCTGGCTGGCT
 CGGGCAGCGGTATCAGCTACTCAAAGGCCGTTACCGTTATCCACAGAAC
 AGGGATAACCGAGGAAGAACATGTGAGCAGGAAAGGCCAGGAA

- continued

RAW SEQUENCE LISTING

CCGTAaaaAGGCCGCGTTGCTGGCCTTTCATAGGCTCCGCCCTGACGA
 GCATCACAAAATCGACGCTCAAGTCAGAGGGCGAAACCCGACAGGACTATAA
 AGATACAGGCTTCCCCCTGGAAGCTCCCTCGCTCGCTCCCTGTTCCGACC
 CTGCCGCTTACCGGATACCTGTCGCCCTTCCCTCGGGAGCGTGGCGCTTT
 CTCATAGCTCACGCTTAGGTATCTCAGTTGGTAGGTGTTGCTCAAGCT
 GGGCTGTGTCACGAACCCCCGTTAGCCGACCGCTGCGCCTTATCCGGTAA
 CTATCGCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCACAGCC
 ACTGGTAACAGGATTAGCAGAGCAGGGTATGTAGGCGGTGCTACAGAGTTCTGA
 ATGGTGCCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCCCTCT
 GCTGAAGCCAGTACCTCGAAAAAGAGTTGGTAGCTTGTATCCGGAAACAA
 ACCACCCCTGGTTTTGGCATGGGTTACGGCAGGAGATTACGCCAGAA
 AAAAGGATCTCAAGAACATCTTGTATCTTCTACGGGCTGACGCTCAAG
 GAACAAAATCTACGTTAAGGGATTGGCATGAGATTATCAAAGATCTTCA
 CCTAGATCCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATATGAGTA
 AACTTGCTCTGACAGTTACCAATGCTTAATCAGTGAGGACCTATCTCAGCGATCT
 GTCTATTCGTTATCCATAGTTGCGTACTC

SEQ ID NO: 33. NUCLEOTIDE SEQUENCE OF PLASMID 456
 GGCGTAATGCTCGCAGTGTACAACCAATTCTGATTAGAAAAACTC
 ATCAGACATCAAATGCAATTATTCATACGAGGATTATCAATACCATATT
 TTGAAAAGCGCTTCTGTAATGAGGAAAACCTACCGAGGCAATTCCATAGG
 ATGGCAAGATCTGGTATCGCTGCGATTCCGACTCGTCCAACATCAATACAC
 CTATTAATTTCCCTCGTCAAAAATAAGGTATCAAGTGAGAAATCACCATGAGT
 ACAGACTGAATCCGGTGAAGAATGGAAAAGCTATGCAATTCTTCCAGACTTGT
 AACAGGCCAGCATTACGCTGTCATCAAAATCACTCGCATCAACAAACCGTTA
 TTCATTGCGATTGCGCTGAGCGAGCGAAAATACGCGATCGCTGTTAAAGGAC
 ATTACAAACAGGAATCAAATGCAACGGCCAGGAACACTGCCAGCGATCAAC
 ATAATTTCACCTGAATCAGGATTCTCTAATACCTGGAATGCTTTCGGG
 GGATCGCAGTGTGAGTAACCATGCGATCAGGAGTACCGATAAAATGCTGAT
 GGTGCGAAGAGGCATAAATTCCGTAGCCAGTTAGTCTGACCATCTCATCTGA
 ACATATTGGCAACGCTACCTTTGCGATGTTAGAAACAACTCTGGCGATCGG
 GCTTACGTTACATACGATAGATGCGACCTGATTGCGGACATTATCGCGAGC
 CCATTATACCCATAAAATCAGCATCTGGAATTAAATCGGGCTCGAGC
 AAAGACGTTCCGTTGAATATGGCTCATAAACCCCTGTTAGTACTGTTATGAA
 GCAAGACGGTCAACATATTGGCTATTGGCCATTGCGATACGTTGATCTATATCAT
 ATATGTCGATATTATGGCTCATGTTCAATATGACCGCCATTGTTGACATTGATTA
 TTGACTAGTTATAATAGTAATCAATTACGGGCTTATTAGTTCATAGCCCATATATG
 GAGTTCCGCGTTACATAACTACGGTAAATGGCCGCCCTGGCTGACGCCAAC
 GACCCCGCCCATGACGTAAATGACGTATGTTCCCATAGTAAACGCCAATAG
 GGACTTCCATGACGTCATGGGTGGAGTTACGGTAAACTGCCCACTTGGC
 AGTACATCAAGTGTATCATGCAAGTCCGGCCCTATTGACGTCATAGCGGT
 AAATGGCCCGCTGGCATTATGCCAGTACATGACCTTACGGGACTTCTACTT
 GCGAGTACATCTACGTTAGTACGCTTACCATGGTATGCGGTTTGGCA
 GTACGACCAATGGCGTGGGAGTACGGTTGACTCACGGGATTTCAGTCTC
 CCCATTGCGTCAATGGAGTTGGCCATTGGCACAATACGGGACTTCCCAA
 ATATGCGTAATAACCCGCCCGTTGACGAAATGGCGTAGGGCTGACGGT
 GGGAGGTCTATAAAGCAGACTCGTTAGTGAACCGTCAGATCGCTGGAGAC
 GCCATCACCGCTGGCTTGGCCATAGAAGACACCGGGGACCGATCCAGCCTCC
 GCGGCGGGAAACGGTCATTGGAAACCGGGATTCCCGTGCCAAAGGTGACTCA
 CGCTCCGAGATCTCGAAGCAGGTATGTACTCTCAGGGTGGCTGGCTTCCC
 CAGTCAGAAGACTCAGGGATTGAGGAGCGCTGTTGGCTCTCTTACATGACC
 TTTGCTTGCTCAACCTGACTATCTCCAGGCTCAGGATCCAGAGTCAGGGT
 CTGTATTTCCTGCTGGCTCAGTTGAGAACAGTAACCCCTGCTCCGAATA
 TTGCTCTCACATCTCGTCATCTCCGAGGACTGGGACCTGTCAGAACAT
 GGCTAGCATTGTTGGAGGCTGGAGTGCAGAGAACGATTCCACCCCTGGCAGGT
 GCTGCTGGCCCTCGTGGCAGGGCAGTCTGGCGCTGGCTCAGAGCTGCC
 GTGGGCTCTCACGCTCAGGCTGCCACTGCGATCAGGAACAAAAGCGTGGATCTG
 TCGGACAGCTGGTTTACCTGAGAACACAGGCCAGGTATTCAAGTCAGC
 AGCTTCCACACCCGCTCTAGCATATGAGCCTCTGAAGAACATGATTCTCAGGC
 CAGGTGATGCTCCAGCCACGACCTCATGCTGCTCCGCTGTCAGAGCTGCC
 AGCTCACGGATCTGTAAGGTGATGGACCTCCAGGAGGCCAGACTGG
 GGACCAACTGCTACGCTCAGGCTGGGCAAGCATTGAAACAGAGGAGTTCTGA
 CCCAAAGAAACTTCAGTGTGGACCTCCATGTTATTCCATGACGTGTTG
 GCAAGTTCACCCCTCAGAGGTGACCAAGTTGATGCTGCTGGACGCTG
 AGGGGCAAACCCACCTGCTGGGTGATTCTGGGGGCCACTTGTGTAATGG
 TGCTGCTTCAAGGTATCACGTCATGGGAGCTGAACCATGTCCTG
 GCCTTCCCTGTCACACCAAGGTGGTCAATTGGGAAGTGGATCAAGAACATC
 GTGGCCAACCCGGATCCCAGACCTGAACTTGTATCTGCTGAAACTGGCAGC
 GATGTGAAAGCAACCCAGGCCAATGGCAAGCGCCGCCGCCGGCTGGCT
 GTGGCTGGGGCGCTGGCTGGCTGGGGCTCTTCTCTCGGCTTCTCTT
 CGGGTGGTTATAAAATCCTCCAAATGAAGCTACTAACATTCTCAAAGCATAATA
 TGAAGCATTGGATGAATTGAAAGCTGAGAACATCAAGAACATTAATT
 TTACACAGATACCACATTAGCAGGAACAGAACAAAATTTCACTGTTG
 CAAGCTGGCTGGGACATTATGAGCTCATCCAAACTACATCTCAATAATT
 ATCAATCCCAAGGAAAGAATTGCGCTGGGATCTGTTGAGCTGGC
 ATTCCTGTTGCTCTACCCAAATAAGACTCATCCAAACTACATCTCAATAATT
 AGATGAAATGAGATTTCACACATCATTATTGAAACCCCTCCAGGATATG
 AAAATGTTCCGATATTGACCCCTTCACTGTTCTCTCAGGAAAGATGCCA

- continued

RAW SEQUENCE LISTING

GAGGGCGATCTAGTGTATGTTAATGCACGAACTGAAGACTCTTTAAATTGGA
 ACGGGACATGAAAATCAATTGCTCTGGGAAAATTGTAATTGCCAGATATGGAAA
 GTTTCTAGAGGAATAAGGTTAAAAATGCCAGCTGGCAGGGCAAAGGAGTC
 TTCTACTCCGACCCCTGCTGACTACTTGTCCCTGGGGTGAAGTCTATCCAGA
 TGTTGGAATCTCTGGAGGGTGTCCAGCTGAAAATATCCAAATCTGAAT
 GGTGCAGGAGACCCCTCACACCCAGTTACCCAGCAAATGAATATGCTTATAGGC
 GTGGAATTGCAAGGAGCTGGTCTTCCAAGTATTCTGTTCATCCAATTGGATAC
 TATGATGCAAGAGCTCTTCAAGGAGGGCTAGCACCACAGATAGCA
 GCTGGAGAGGAAGTCTCAAAGTGCCTACATGTTGACCTGGCTTACTGGAAA
 CTTTCTACACAAAAAGTCAAGATGCACATCACTTACCAATGAAGTGACAAGAA
 TTACAAATGTGATAGGTAACCTCAGAGGAGCAGTGGAAACAGACAGATATGTCAT
 TCTGGAGGCTCAGGGGACTCATGGTGTGTTGCTGTTGACCTCAGACTGG
 AGCAGCTGTTGTCATGAAATTGTGAGGAGCTTGGAAACACTGAAAAGGAGGG
 TGGAGACCTAGAGAACAAATTGGTGTGCAAGCTGGGATGCGAGAAGAATTGGTC
 TTCTGGTTCTAGTGTGGGAGGAGATTCAGACTCTTCAAGAGGCGTGG
 CGTGGCTTATAATTAACTGTCATCATATAGAAGGAAACTACACTCTGAGAGTTG
 ATTGTAACCCGCTGATGTCAGCTTGGTACACAACTAACAAAGAGCTAAAAG
 CCTGTGAGGGCTTGAAGGCAAATCTCTTATGAAAGTTGACTAAAAAGTC
 CTCCCGAGAGTCCTGACTGGCATGCCAGGATAAGCAAATTGGATCTGGAATG
 TTTGAGGTGTTCTCAACGACTTGGGATATTGCTTCAAGGCAGAGCAGGTAACTA
 AAAATTGGAAACAAACAAATTCAAGGGCTTCAACTGTTATCACAGTGTCTATGAA
 ACATATGAGTTGGTGGAAAAGTTTATGATCCAATGTTAAATATCACCTACTGT
 GCCCAGGTTGAGGGGGATGGTGTGAGCTGGCAATTCCATAGTGTCTCC
 TTTGATTGTCAGGATATTGCTGAGTTTAAGAAGATGCTGACAAAATTCTACAG
 TTTTCTGCACTAAAGAATTTCAGAAATTGCTTCAAGTTCAGTGTGAGAGACTCC
 AGGACTTTGCAAAAGCAACCCAACTAGTATTAGAATGATGATGATCAACTCATG
 TTCTGGAAAAGGCAATTGATCCATTAGGGTACAGACAGGCTTTTATAG
 GCATGTCATCTGCTCAAGGCCACAAAGTATGCGAGGGACTATTCCA
 GGAATTATGATGCTCTGGTATTTGAAAGCAAAGTGGACCCCTTCAAGGGCTG
 GGGAGAAGTGAAGAGCAGATTATGTTGAGGCTTCAAGTGCAGGCAGCTGC
 AGAGACTTTGAGTGAAGTGGCTTAAAGGATCTGACCCCCAACTACGTTACTGGCGAA
 GCCGTTGGATAAGGCCGGTGTGCGTTGCTATATGTTATTTCACCATATTG
 CCGCTTTGGCAATGTGAGGGCCGGAAACCTGGCCCTGTCCTTGTGAGGC
 ATTCCTAGGGGCTTCCCTCTGCCAAAGGAAATGCAAGGCTGTGTAATGTCG
 TGAAGGAAGTCAGTCTCTGGAAAGCTTGTGAGGAAACAAACGCTGTAGCGAC
 CCTTGAGGAGCGGAACCCCCCCTGGGAGACAGGTGCCTCTGGGCAAAA
 GCCACGTGATAAGATACACCTGCAAGGGGGCAACACCCAGTGCACGTTG
 GAGTTGGATACTGTGAGGAAAGGTCAAATGGCTCTCTCAAGCGTATTCAACAG
 GGCGTGAAGGTGCCAGAAAGGTACCCATTGATGGGATCTGATCTGGGCT
 CGGTGACATGCTTACATGTTAGTCAGGGTAAAGGACTAGGCTTAGGCCCCC
 GAACCCAGGGGACGTGGTTCTGGAAAACACGATGATAATGGCAGCAA
 GCGTGTGCTGTGCTGGCTGTGATGCCAGGCTGGCCCTGCGAGGCCAGTGC
 CCTGCTGCTGACTCTGCAAGGCCAGGTGAGCAACGAGGACTGCCCTGAGGT
 GGAGAACTGCAAGGCCAGCTGGGGAGCGTGTGGACCGCGCAGCGCAG
 TTGGCCTCTGACCGTCATCAGCAAAGGCTGCAAGCTTGAATGCTGGATGACTC
 ACAGGACTACTACGTGGCAAGAGAACATCACGTGCTGTGACACCGACTTGT
 CAAGCAGGGGGCCATGGCTGCGGCCATCCCTGGCGCTGC
 TCCCTGCACTGGCTGCTGTGCTGGGACCCCCCAGCTATAAGGGATCTGGC
 CCTAACAAAACAAAAGATGGGTTATTCCCTAAACTTATGGTTACGTAATTGG
 AAGTTGGGGACATTGCAAAAGATCATATTGTAACAAAGATCAAACACTGTTTA
 GAAAATCTCTGAAACAGGCTATTGATTGAGGAAAGTATGTCAGGAAAGGATTGGG
 CTTTGGGCTTGTGCTGCTTACACATGTTGAGGATATCTGCCCTAATGCTTT
 GTATGATGATAACAGCTAACAGGCTTACTTCTGCCCTAAGGCT
 TTCTAAGTAAACAGTACATGAACCTTACCCGTTGTCGGCAACGGCTGGTCT
 GTGCCAAGGTGTGCTGAGCACAACCCCACTGGCTGGGCTGGGATAGGCCA
 TCAGCGCATGCGCATGGGACCTTGTGGCTCTGGCGATCCATACTGCCGAAACTC
 CTAGCGCTTGTGCTGAGCCGGTCTGGAGAAAGCTCATAGGAACGTACA
 ATTCTGCGTCCCTCGCGGAAATATACATGTTGATCACGTATGATCTTTT
 CCTGCGAAACAAATTATGGGACATCATGAAGCCCTTGGACATCTGACTCTGG
 CTAATAAAGGAAATTATTTCTTGTGCAATAGTGTGTTGGAAATTGGTGTCTCTC
 ACTCGGAAGGAAATTCTGCAATTATGATGTCGCAACGCCGGGAGGGCGTT
 TCGTATTGGGCGCTTCCGCTCTCGTCACTGACTCGTGTGCGCTGGTCT
 TCGGCTGCGGGAGCGTACAGCTCACTAACGGGTAATACGGTTATCCAC
 AGAATCAGGGATAACGGAGAAAGAACATGTGAGCAAAAGGCCAGCAAAGGC
 CAGGAAGCTGGGCTGGCTGCTGGGTTTCCATAGGCTCCGGGGGG
 TGACGAGCATCACAAAATCAGCGCTCAAGTCAGGGTGGGAAACCCGACAGG
 ACTATAAAGATACCGGGCTTCCCGTGGAGGCTCCCTCGTGTGCGCTCCGT
 CGGACCCCTGCCCTACCGTACCTGTCGCCCTTCTCCCTCGGGAAAGGGT
 GCGCTTCTCATAGTCAGCTCAGCTGAGGTATCTCAGTCGGTGTAGGTCGTC
 CCAAGCTGGGCTGTGTCAGCAAGGCCGGCTTCAAGGCCGACGGCTGCCCTAT
 CGGTAACATCTGCTTGTGAGTCCAACCCGGTAAGACACGACTTATGCCACTGGC
 AGCAGCCACTGGTAACAGGATTAGCAGAGCAGGGTATGAGGGCTGCTACAGA
 GTCTGAGTGTGCTGAGGCTTAACCTACGGTCAACTAGAAGAACAGTATTGGTATCT
 GCGCTGCTGAGGCTTAACCTCGGAAAAGAGTTGGTAGGCTTGTGATCCGG
 CAAACAAACCCGGCTGGTAGGGTGGTTTTGTTGATCAAGCAGCAGATTACG
 CGCAGAAAAAAAGGATCTCAAGAACATCCTTGTGATCTGGGCTGACG

- continued

RAW SEQUENCE LISTING

CTCAGTGGAACGAAACTCACGTTAAGGGTTGGTCATGAGATTATCAAAAAG
 GATCTCACCTAGATCCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATA
 TATGAGTAAACTGGTCTGACAGTACCATGCTTAATCACTGAGGCACCTATCTC
 AGCGATCTGTCTATTCGTTACATGGCTGACTC

SEQ ID NO: 34. NUCLEOTIDE SEQUENCE OF PLASMID 457
 GGCGTAACTGCTGCCAGTGTACACCAAATTACCAATTCTGATTAGAAAAACTC
 ATCGAGCATCAAATGAAACTGCAATTATTCATATCAGGATTATCAATACCATATT
 TTGAAAAGCCCTTCTGTAATGAAGGAAAACCTACCGAGGCAGTTCCATAGG
 ATGGCAAGATCTGGTATCGCTCGGACTCGTCCACATCAATACAAC
 CTATTAACTTCCCTGCTGAAATAAGGTTATCAGGATGAGAAATCACCAGTGAAGTG
 ACAGACTGAATCCGGTGAAGATGCAAAAGCTTATCGTCACTTCAGACTTGTGTC
 AACAGGGCAGCCATTACGCTGTCATCAAAATCACTCGCATCAACCAAACCGTTA
 TTCATTGCTATTGCGCTGAGCGAGACGAAATACGCGATCGCTGTTAAAGGAC
 ATTACAAACAGGAATCAAATGCAACCGGCCAGGAACACTGCCAGCGCATCAAC
 ATATTTTCACTGAATCAGGATATTCTCTAATACCTGGAATGCTGTTTCCGG
 GGATCGCAGTGTGAGTAACTGTCATCAGGAGTACGGATAAAATGCTGAT
 GGTGCGGAAGAGGCATAAATTCCGTAGCCAGTTAGTCTGACCATCTCATCTGA
 ACATATTGGCAACGCTACCTTTCGCTATGTCAGAAACACTCTGGCGCATCGG
 GCTTCCCATAACATCGATAGATTGCGCACCCTGATTGCGCAGATTATCGCGAGC
 CCATTATACCCATAAAATCAGCATCGATGTTAAATCGGGCCTCGAGC
 AAGACGTTCCCGTGTGAATATGGCTCATAAACACCCCTGTATTACTGTTATGAA
 GCAGACAGGTAACTATTGGCTATTGGCATTGCTAACAGTGTATCTATATCAT
 ATATGTCATTTATATTGGCTCATGTCATGTCATGACCCATGTTGACATTGATTA
 TTGACTAGTTAAATAGTAATCAATTACGGGCTTATTAGTTCATAGGCCATATATG
 GAGTTCCCGCTTACATAACTACGGTAATGGCCGCTGGCTGACGCCAAC
 GACCCCCGCCATTGAGCTAAATAATGACGTATGTTCCCATAGTAACGCCAATAG
 GGACTTCCATTGAGCTCAATGGTGGAGTTACCGGTAACCTGGCCACTTGGC
 AGTACATCAAGTGTATCATGCGCAAGTCCGGCCCTATTGACGTCATGACGGT
 AAATGGCCCGCCTGGCATTGCGCAGTACATGACCTTACGGGACTTCTACTT
 GCGAGTACATCACGCTTACGTTACGCTCATGCGTATTACCATGGTGTGCGGTTGGCA
 GTACACCAATGGCGTGGATAGCGGTTTGACTCACGGGAGTTCCAAGTCTCAC
 CCCATTGACGTCATGGAGTTGTTTGGCACCATAACCGGACTTCCAA
 AATGTCGTAATAACCCGCCCGTGTACGCAAATGGCGTAGGCGTAGCGGT
 GGGAGGTCTATAAAGCAGAGCTGTTAGTGAACCGTCAGATCGCTGGAGAC
 GCCATCACGCTTTCGCTTCCATAGAAGACACCGGGACCGATCCAGCCTCC
 GGGCGGGAAAGGTGCAATTGGAAACCGGGATTCCCGTGCAGAGTGAATCA
 CCGTCGGATCTCAGCAAGCAGGTATGTACTCTCAGGGTGGCCTGGCTTCCC
 CAGTCAGACTCAGGGATTGAGGACGCTGTGGCTCTCTCTACATGTAAC
 TTTTGCTGCCATTGCGACTATCTCCAGGTCAAGGATCCAGAGTCAGGGT
 CTGTAATTTCCTGCTGGCTCCAGTCAAGGAACTGCTGACCTGGCTGACCC
 TTGCTCTCATACATCTCGCAATCTCGCGAGGACTGGGACCTGTGACGAAACAT
 GCTGAGTATTGCGGAGGCTGGAGTGTGAGAAGACCTTCCAAACCTGGCAGGT
 GCTTGCTGCCATTGCGCAGGCGACTCTGGCGCAGGATCTGGTGCACCCCA
 GTGGGCCTCACGGCTCAGGCTGGCAGGATCTGGCGCAGGCTGGTGG
 TCGGCACAGCTGTTTCACTGTAAGACACAGGCCAGGTATTGAGTCAGCC
 AGCTTCCCACACCGCTCTACGATATGAGCCTCTGAAGAATCGATTCTCAGGC
 CAGGTGAGTGCAGGCTGAGGTCTGGGAGGCTGGAGTGTGAGGCCCTGCG
 AGCTCAGGATGCTGTGAGGTCTGGGAGGCTGGAGTGTGAGGCCAGGACTGG
 GGACCACTGCTACGCCCTACGGCTGGGCACTGGACATTGAACCAAGAGGAGTCTGA
 CCCCAAGAAACTTCAGTGTGGACCTCCATGTTATTCCATGACGCTGTGTC
 GCAAGTTCACCCCTGAGGTGACAGGCTGGGAGGCTGGAGTGTGAGGCC
 AGGGGGCAAAAGCAGGCTGGGAGGCTGGGAGGCTGGGAGGCTGGAGCAGG
 GATGTTGGAAAGCAACCCAGGCCAATGGCAAGCGCGCCGCGCCTGGCT
 GTGCGCTGGGCGCTGGCTGGGCTGGGTTCTCTCTCGGCTTCTCT
 CGGGTGGTTATAAAATCCTCAAATGAAGCTACTAACATTACTCAAAGCATATA
 TGAAGCATTTGGATGAAATTGAAAGCTGAGAACATCAAGAAGTCTTATATAATT
 TTACACAGTACACACATTAGCAGGAACAGAAACAAACTTCACTGCAAAGCAA
 ATTCATCCAACTGGAAAGAATTGCGCTGGGATTCTGTTGAGCTGGCATTATG
 TGTCCTGTTGCTCTACCCAAATAAGACTCATCCAACATACATCTCAATAATTAA
 AGATGAAATGAGATTTCACACATCATTGTAACCACTCTCCAGGATATG
 AAAATTTGCTGACCTTCAAGGCTTCAAGGCTGGGAGGCTGGAAATATCCTAA
 GAGGGCGATCTAGTGTATGTTACTATGCAAGCAACTGAAGACTTCTTTAAATTGGA
 ACAGGGCATGAAATCAATTGCTCTGGGAAATTGTAATTGCGAGATATGGAAA
 GTTTTCAGAGGAAATAAGGTTAAAGCTGGCCAGCTGGCAGGGCCAAGGAGTC
 TTCTCTACTCCGACCCCTGCTGACTACTTGTGCTCTGGGAGTCTATCCAGA
 TGTTGGAATCTCTGGAGGGTGTCCAGCTGGAAATATCCTAAATCTGAAT
 GGTGCGAGGAGACCCCTCTCACACCCAGGTTACCCAGGAAATGAATATGCTTATAGGC
 GTGGAATTGCAAGGGCTGGCTTCCCAAGTATTCTGTTGACATCCAATTGGATAC
 TATGATGACAGAAGCTCTAGAAAAATGGGGTGGCTCAGCACCCAGATAGCA
 GCTGGAGAGGAAGTCTCAAAGTGCACATCCACTACCAATGAAGTGACAGAAGAA
 CTTTCTACACAAAAAGTCAGATGACATCCACTACCAATGAAGTGACAGAAGAA
 TTACAAATGTGATAGGTACTCTCAGAGGAGCAGTGGAAACGAGACAGATATGTCAT
 TCTGGGAGGTCACCGGACTCATGGTGTGGTATTGACCTCGAGTGG

- continued

RAW SEQUENCE LISTING

AGCAGCTGTTGTCATGAAATTGTGAGGGAGCTTGGAACACTGAAAAAGGAAGGG
 TGGAGACCTAGAAGAACATTGTTGCAAGCTGGGATGCAGAAGAATTGGTC
 TTCTTGTTCTACTGAGTGGGAGAGGAGATTCAAAGACTCCTTAAGAGCGTGG
 CGTGGCTTATAATTAAATGCTGACTCATATAAGAAAACACTACACTTGAGAGTTG
 ATTGTACACCCTGTAGTACAGCTTGGTACACAACCTAACAAAAGAGCTAAAAG
 CCCGTATGAAGGCTTGTAGGCAAATCTCTTATGAAAGTGGACTAAAAAGTC
 CTCCCCAGACTGGCATGCCAGGATAAGCAAATTGGGATCTGGAATG
 TTTGAGGTGTTCTTCAACGACTTGGATTGCTCAGGCAGAGCACGGTATACTA
 AAAATTGGAAACAAACAAATTCAAGCGCTTCCACTGTATCACAGTGTATGAA
 ACATATGAGTGGTGGAAAAGTTTATGATCAAATGTTAAATATCACCTACTGT
 GCCAGGCTGGGAGGAGGATGGTGTGGACTGGCAATTCCATAGTGTCCC
 TTTGATTGTCAGATTATGCTGTTAAAGAAGTATGCTGACAAAATCTACAG
 TATTCTATGAAACATCACAGGAAATGAAGACATACAGTGTATATTGATTCACT
 TTTTCTGCAGTAAAGAATTACAGAAATTGCTTCAAGTTCAGTGAGAGACTCC
 AGGACTTGTGAAACAAACCCAAATAGTATTAGAAATGATGAATGATCAACTCATG
 TTCTGGAAAGGCAATTATTGATCCATTAGGGTACCCAGACAGGCTTTTATAG
 GCATGTCATCTGTCACAGCCACAAAGTATGCAAGGGAGTCATCCCCA
 GGAATTATGATGCTCTGTTGATATTGAAAGCAAAGTGGACCCCTCCAAGGCCG
 GGGAGAAGTGAAGAGACAGATTATGCTGAGCCTTCACAGTGCAAGGCAGCTGC
 AGAGACTTGTGAGTAGCGGAGATCCGAAGGGTAGGGGTCATTATTGACCTGT
 GGAGATGTCAGGAAACCCAGGACCCGAAGCAAGGCTGTGCTGTTGCCCTG
 TTGATGGCAGGCTTGGCCCTGCAGCAGGACTGCCCTGCTGTGACTCTGC
 AAAGCCAGGTGAGAACCGAGACTGCCCTGAGGTGGAGAACCTGCACCCAGCTG
 GGGGAGCAGTGTGACCTGGCAGCTCCGCGCAGTGGCCTCTGACCCGTCAT
 CAGCAAGGGCTGAGCTTGAACGTGCGTGTGACTCACAGGACTACTCGTGGG
 CAAGAAGAACATCACGTGCTGTGACACCGACTTGTGCAACGCCAGCGGGGCCA
 TGCCCTGCAGGGCTGCCCATCTGCCCTGCTGCCACTCGGCCGTGCT
 GCTCTGGGACCGGGCAGCTAGAGATCTGGCCCTAACAAAACAAAAGAT
 GGGGATTCTCTTAAACTTATGGGTTACGTAATTGAAAGTGGGGGACATTGCC
 ACAAGATCATATTGTACAAAGATCAAACACTGTTTAGAAAACCTCTGTAACAG
 GCCTATTGATTGAAAGTATGCTCAAAGGATTGTTGGCTTTGGGCTTGTGCTC
 CATTACACAAATGTGGATATTGCTTAACTGCTTGTATGCTGATGATACAGCT
 AACACAGGCTTCACTTCTGCCAACTTAAAGGCTTTCTAAGTAAACAGTACAT
 GAACCTTACCCGTTGCTGCCAACGGCTGGTCTGTGCGCAAGTGTGCTGAC
 GCACCCCTACTGGCTGGGCTTGGCCATAGGCCATCAGGGCATCGGTGAAACC
 TTGTGGCTCTGCCATACTGCCGAACTCTAGCCGTTGCTGCTGCT
 CGAGCGCTGGAGCAAAGCTCATAGGAACCTGACAATTGTGCTCTCGC
 GGAATATACATGTTGATACGTATGATCTTCTCCCTGCAAAATTATG
 GGGACATCATGAGGCCCTTGAGCATTGACTCTGGCTAATAAAGGAAATTATT
 TTCAATTGCAATAGTGTGGGATTTTTGTGCTCTCACTCGGAAGGAAATTCTGC
 ATAATGAATGCCAACGCCGGGGAGAGGGCTTGGCTATTGGGCGCTCTT
 CGCCTTCTCGCTCACTGACTCGCTGCGCTCGTGTGCGTGTGCGGAGCG
 TATCAGCTCACTCAAAGCGTAATACGGTTATCCACAGAACCTAGGGGATAACGC
 AGGAAAAGAACATGTGAGCAAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGC
 CGCGTGTGGCTTCCCATAGGCTGCCCTTGACGAGCATCACAAAAT
 CGACGCTCAAGTCAAGGGCTGCCAACCGACAGGACTATAAGATACAGGG
 TTCCCCCTGGAAGGCTCTCGTGTGCGCTCTCTGTTCCGACCTGCCGCTTACCG
 GATACTGTCCTGGGAAAGCTCTCGTGTGCGCTTCTCATAGCTCAG
 CTGTAGGTATCTCAGTCGGTGTAGTCGCTGCCAAAGCTGGCTGTGCGAC
 GAACCCCGTCAAGACAGACTTATGCCACTGGCAGCAGCCACTGGTAAACAGGA
 TTAGCAGAGCAGGTTAGTGGCGGTGCTACAGGTTCTGAAGTGTGGCCCTA
 ACTACGGCTACATAGAAGAACAGTATTGGTATCTGCGCTGTGAGCAGT
 TACCTGGAAAAGAGTTGGTAGCTTGTGATCCGGCAAACAAACCCAGCTGG
 AGCGGTGTTTTTGTGCAAGCAGCAGATTACGCCAGAAAAAGGATCTC
 AAGAAGATCTTGTGATCTTCTACGGGCTGACGGCTACTGGAAACGAAAACCTC
 ACGTTAAGGGATTGGTCACTGAGATTATCAAAGGATCTCACCTAGATCTTT
 TAATTAATTAATGAAGTTAAATCAATCTAAGTATATAAGTAAACTTGGCTG
 ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTAGCGATCTGTATTG
 TCATCCATAGTGCCTGACTC

SEQ ID NO: 35. NUCLEOTIDE SEQUENCE OF PLASMID 458
 GGCGTAATGCTGCCAGTGTACAACCAATTAAACCAATTCTGATTAGAAAAACTC
 ATCGAGCATAAATGAAACTGCAATTATTATTCATATCAGGATTATCAATACCAATT
 TTGAAAAGGCTTCTGTAATGAAAGGAAAACACTACCCGAGGAGTTCATAGG
 ATGGCAAGGATCTGGTATCGCTGCGATTCCGACTCGTCAACATCAACAAAC
 CTATTAACTTCCCTCGTCAAAATAAGGTTATCAAGTGAAGAAATCACCATGAGT
 ACGACTGAATCCGGTGAGAATGCCAAAGCTTATGCTATTCTCAGACTTGT
 AACAGGCCAGCATTACGCTGTCATCAAATCACTCGCATCAACCAACCGTTA
 TTCATTGCTGATGCCCTGAGCGAGACGAAATACCGCGATCGCTTAAAGGAC
 AATTACAAACAGGAATCAAATGCCAACCGGCCAGGAACACTGCCAGCGCATCAAC
 AATATTTCACCTGAATCAGGATTCTCTTAATACCTGAAATGCTGTTCCCG
 GGATCGCAGTGGTGAATGCCAGGACTACGGATAAAATGCTGAT
 GTCGGAAGAGGCCATAATTGGTCACTGGCAGGACTTGTGCTGACCATCTCATG
 ACATCATTGGCAACGCTACCTTGCCATGTTGAGAACACTCTGGCGCATCGG
 GCTTCCCATACATGATAGATTGTCGACCTGATTGCCGACATTATCGCGAGC
 CCATTATACCCATATAATCAGCATCCATTGGAATTAAATCGCGGCTCGAGC

- continued

RAW SEQUENCE LISTING

AAGACGTTCCCGTTGAATATGGCTCATAACACCCCTGTATTACTGTTATGTAA
 GCAGACAGGTGACAATATTGGCTATTGGCATTGCTACGTGTATCTATATCAT
 ATATGTACATTATATTGGCTCATGTCAAATGACCGCCATGTCAGATTGATTA
 TTGACTAGTTATAATAGTAACTCAATTACGGGTATTAGTCATAGCCCCATATG
 GAGTTCCCGCTTACATAACTACCGTAAATGCCCGCCCTGGCTGACGCCAAC
 GACCCCGGCCATTGACGTCAATAATGACGTATGTTCCATAGTAACGCCAATAG
 GGACTTCCATTGACGTCAATGGGTGGAGTTACGGTAAACTGCCACTTGGC
 AGTACATCAAGTGTATCATGCCAAGTCCGGCCCTATTGACGTCAATGACGGT
 AAATGCCCGCCCTGGATTATGCCAGTACATGACCTTACGGACTTTCTACTT
 GGCAGTACATCTACGTATTAGTCATCGTATTACCATGGTGTGCGGTTTGCA
 GTACACCAATGGGTGGATGACTCACGGGATTCCAAGTCTCACC
 CCCATTGACGTCAATGGAGTTGTTTGCCACAAAATCAACGGGACTTCCAA
 AATGTCGTAATAACCCGCCCGTTGACGCAAATGGCGTAGGCGTGTAGGT
 GGGAGGTCTATAAAGCAGCTCGTTAGTGAACCGTCAGATCGCTGGAGAC
 GCCATCACGCTGGCTTACAGTCCAGGAACTGCCAGCTCC
 CGGGCCGGAAACGGTGCATTGAAACGCCGATTCCCCTGCCAGAGTCA
 CGTCCGGATCTAGAAGCAGGTATGACTCTCAGGGTGGCTGGCTTCCC
 CAGTCAGAAGCTCAGGGATTGAGGGACGCTGTTGGCTCTTCCTTACATGACC
 TTTGCTTGCCTCAACCTGACTATCTCCAGGTAGGATCCAGAGTCAGGGT
 CTGTTATTTCTCTGGCTGGCTCCAGTTCAGGAAACAGTAAACCTGCTCCGAATA
 TTGCTCTCATCTCGTCACTCTCCGAGGACTCGGGACCTGTGACAAACAT
 GGCTAGCATTGTTGGAGGCTGGGAGTGCAGAGAACGATTCCAAACCTGGCAGGT
 GCTTGGCCCTCTCGTGGCAGGGCAGTCTGGCGCTGTGTTCTGGTGCACCCCA
 GTGGCTCTCACAGCTGCCCACCTGCATCAGGAAACAAAAGCCTGATCTGCTGG
 TCGGCAAGCTGGTTTACCTGAAGACACAGGCCAGGTATTCAAGGTCAAGCAC
 AGCTTCCCACACCGCTCTACGATATGAGCCTCTGAAGAATCGATTCTCAGGC
 CAGGTGATGACTCCAGGCCACGACTCTGCTCCGCTGTCAAGAGCCTGCG
 AGCTCACGGATCTGTAAGGTCTGGACCTGGCAGGACCTGGCAGACTGG
 GGACCACTCTGCTACGCCAGGGCACATTGAACCAAGAGGAGTTCTGA
 CCCCAAGAAAACCTCAGTGTGGACCTCCATGTTATTCCAATGACGTGTGTC
 GCAAGTTCACCCCTCAGAAGGTGACCAAGTTCAGCTGTGTGCTGGAC
 AGGGGGAAAGAACCTGCTCGGGTGAATTCTGGGGGCCACTTGTCTGTAATGG
 TGTGCTTCAAGGTATCACGTATGGGGCAGTGAACCATGTCCTGCCAAGAAG
 GCCTCCCTGTACACCAAGGTGGCTTACCGGAAGTGGATCAAGGACACCATC
 GTGGCCAACCCGGATCGAAGGTAGGGGTCATTATTGACCTGTGGAGATGTC
 GAAGAAAACCCGGCTGAGCAAGGCTGTGCTGGCTTGGCTGTGATGGCA
 GCGCTGGCCCTGCAAGGCCAGCTGCCCCTGTGCTACTCTGCAAAGGCCAG
 GTGAGCAACGAGGACTGCCAGGTGGAGAACGTCACCCAGCTGGGGAGCA
 GTGCTGGACCGCGCATCCGGCAGTGGCTCTGACCGTCATCAGCAAAGG
 CTGCGCTTGAACCTGCTGGATGACTCACAGGACTACTACGTTGGCAAGAAGAA
 CATCACCTGCTGTGACACCAGCTGTGCAACGCCAGGGGCCATGCCCTGCA
 GCGGCTGCCCATCTTGGCTGCTCCCTGCACTGGCTGCTGTCTGGGG
 ACCGGCCAGTAGGATCCAGACCTGAACTTGTCTGTAACCTGGCAGG
 CGATGCGGAAACCAACCCAGGCGCAACGGCCAAATGGCAAGCGCGCCGGCGTGGC
 TGTGCGCTGGGGCCTGGCTGGGGGGCTCTCTCTCTCGCTTCCCT
 TCGGGTGTCTTAAATCTCCAATGAAGCTACTAACATTACTCCAAGCATATA
 ATGAAAGCATTTTGATGAATTGAAAGCTGAGAACATCAAGAAGTCTTATAATA
 TTACACAGATCACATTAGCAGGAACAGAACAAAACCTTCAGCTGCAAAGCA
 ATTCAATCCAGTGGAAAGATTGGCTGGATTCTGTTGAGCTGGCAATTATG
 ATGTCCTGTTCTACCCAAATAAGACTCATCCAAACTACATCTCAATAATTATG
 AAGATGGAATGAGATTTCACACATCATTATTGAAACCACCTCTCCAGGATAT
 GAAAATGCTGGATATTGTAACCTTCTGGTACTGCTTCTCCCTCAAGGAATGCC
 AGAGGGCAGTCTAGTGTATGTTAATCTGACGCAACTGAAGACTCTTTAAATTGG
 AACGGGACATGAAAATCAATTGCTGGAAAATTGTAATTGGCAAGATATGGAA
 AGTTTCAGAGGAAATAAGGTTAAAATGCCAGTGGCAGGGGAAAGGAGTC
 ATTCCTACTCCGACCCCTGCTGACTACTTCTCTGGGTGAAGTCTCATCAG
 ATGGTTGGAAATCTCTGGAGGTGGTCCAGCGTGGAAATATCTAAATCTGAA
 TGGTGCAGGAGACCCCTCACACCGAGTTACCCAGCAAATGAATAATGCTTATAG
 CGTGGAAATTGCGAGGGCTGTGGCTTCCAAGTATTCTGTTCATCCAATTGGATA
 CTATGATGCAAGAACGCTCCCTAGAAAAAATGGCTGGCTCAGCACCCACAGATAGC
 AGCTGGAGAGGAAGTCTAAAGTGCCTACATTGTTGGACCTGGCTTACTGGAA
 ACTTTCTACACAAAAGTCAAGATGCCATCCTACTCAACCATGAAGTACAAGA
 ATTTCACATGTGATAGGTACTCTCAGAGGAGCAGTGGAAACAGACAGATATGTC
 TTCTGGGAGGTACCCGGACTCATGGGTGTTGGTGTATTGACCTCAGAGTG
 GAGCAGCTGTGTTGATGAATTGTCAGGAGCTTGGAAACACTGAAAAGGAAGG
 GTGGAGAGCTAGAAGAACATTGTTGCAAGCTGGGATGCAAGAAGAATTGGT
 CTCTTGGCTTACTGAGTGGCAGAGGAGATTCAAGACTCTCTAAGAGCTG
 GCGTGGCTTATAATGTCAGTCTATAGAAGGAAACTACCTGAGAGT
 GATTGTACACCGTGTACAGCTGGTACACAACCTAACAAAGAGCTGAAAAA
 GCCCTGATGAAGGCTTGAAGGCAATCTTTATGAAAGTGGACTAAAAAAGT
 CCTTCCCGAGGTCAGTGGCATGCCAGGATAAGCAAATTGGGATCTGGAAATG
 ATTTGAGGTGTCTTCAACGACTGGAAATTGCTTCAGGAGCACGAGTATACT
 AAAAATGGGAAACAAACAAATTCAAGCGGTATCCACTGTATCACAGTGTATGAA
 AACATATGAGTTGGTGGAAAATTGATGTCATGTTAAATACTCACCTCACTG
 GGCCAGGTTGAGGAGGGATGGTGGTGGCAATTCTCATGTGCTCC
 TTTGATTGTCAGGATTATGCTGTAGTTAAAGAAAGTATGCTGACAAAATCTACAG
 TATTCTATGAAACATCCACAGGAAATGAAGACATACAGTGTATCTGATTCACT

- continued

RAW SEQUENCE LISTING

TTTTCTGCAGTAAAGAATTACAGAAATTGCTTCAAGTTCAAGTCACTGAGAGACTCC
 AGGACTTTGACAAAAGCAACCCAAATAGTATTAAAGAATGATGAATGATCAACTCATG
 TTCTCGAAAAGAGCATTATTGATCATTAGGGTACCGAGACAGGCCCTTTTATAG
 GCATGTCACTATGCTCCAAGCAGCCACAAAGATGAGTGCAGGGAGTCATTCCA
 GGAAATTATGATGCTCTGTTGATAATTGAAAGCAAAGTGACCTTCAGGCTG
 GGGAGAAGTGAAGAGACAGATTATGTTGCAGGCCACAGTCAGGAGCTG
 AGAGACTTTGAGTGAAGTAGCTTAAAGATCTGGGCTAACAAAACAAAAGATG
 GGTTTACCTCTTACCTATGGTACAGTAAAGATCTGGGCTTGGAGTGGGGACATTGCCA
 CAAGATCATATTGACAAACACTGTTTAGAAACACTTCTGAAACAG
 GCCTATTGATTGAAAGTATGCTAAAGGATTGTTGGCTTTGGCTGCTC
 CATTACACAATGAGTACCTGCTTAAATGCTTGTATGCACTGATAACAGT
 AAACAGGCTTCACTTCACTTCCCAACTTACAGGCCCTTCTAAGTAACACAT
 GAACCTTACCCCGTGTGCTCGCAACGGCTGGCTGTGCCAAGTGTGAC
 GCAACCCCCTGGCTGGGCTGGCATAGGCCATAGCGCATGCGTGGAAACC
 TTGTGGCTCTGGATCCTGCGGACTCTAGCGGCTGGTGTGCTC
 GCAGCCGCTGGAGCAAAGCTCATAGGAAGTACAATTCTGTCGCTCTCGC
 GGAATATACATGTTGATCACGTATGATCTTTCCCTCTGCCAAAATTATG
 GGGACATCATGAAGCCCCCTGAGCATCTGACTCTGGTAATAAAGGAATTATT
 TTCAATTGCAATAGTGTGTTGGATTTTTGTCGCTCTCACTCGGAAGGAATTCTGC
 ATTAAATGCAATGGCCAACCGCAGGGAGGGGGAGGGGGTGGTGTATTGGCGCTCTT
 CGCGCTCTCGCTCACTGACTCGCTCGCGCTGGCTGCGGAGCG
 TATCAGCTCACTAAAGCGGTAATACGGTTATCCACAGAATCAGGGATAACGC
 AGGAAAGAACATGTGAGCAGGAAAGGCCAGAAAAGGCCAGGAACCGTAAAAGGC
 CGCGTGTGGCTGGGTTTCCATAGGCTCGGCCCCCTGACGAGCATTCAAAAAAAT
 CGACGCTCAAGTCAGAGGTGGCAAACCCGACAGGACTATAAAGATACAGGCG
 TTCCCCCTGGAAGGCTCTCGTGCCTCTCTGTGCGCTACAGGTTCTCATAGCTCAG
 GATACTGTCGCCCCCTCTCCCTCGGAAGCGTGGCGCTTCTCATAGCTCAG
 CTGAGGTATCTCAGTTGGTGTAGGTCGTTGCTCAAGCTGGCTGTGAC
 CCAACCCGGTAAAGACAGCACTATGCCACTGGCAGCAGGACTGGTAACAGGA
 TTAGCAGAGGGTATGAGTGGCTGAGGTTCTGAAGTGTGGCTA
 ACTACAGCTCACAGAAGAACAGTATTGGTATCTGCGCTGCTGAAGCCAGT
 TACCTTGGAAAAGAGTTGGTAGCTTGTATCCGCAAACAAACCCAGCTGGT
 AGCGGGTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTC
 AGGAAGATCTCTGATTTTCTACGGGCTGACGCTCAGGGAAAGGAAACTC
 ACGTTAAGGGATTATGGTCATGAGATTACATCTAAAGTATATGAGTAAACTGGTCTG
 ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTAGCGATCTGTATTCTG
 TCATCCATAGTGGCTGACTC

SEQ ID NO: 36. NUCLEOTIDE SEQUENCE OF PLASMID 459
 GCGCTATGCTGCCAGTGTACAACCAATTAAACCAATTCTGATTAGAAAAACTC
 ATCGAGCATAAATGAAACTGCAATTATTATTCATATCAGGATTATCAATACCATATT
 TTGAAAAGCCCTTCTGTAATGAAAGGAAAAGACTCACCGAGGCAAGTCCATAGG
 ATGCAAGATCTGGTATCGCTGCGATTCCGACTCGTCAACATCAACAAAC
 CTATTAACTCCCTCGTCAAAATAAGGTATCAAGTGGAAATCACCATGAGTG
 ACGACTGAATCCGGTGAGAATGGCAAAGCTATGCAATTCTCGCATCAACCCAGGTTA
 AACAGGGCAGGCAATTACGCTGTCATCAAAATCACTCGCATCAACCCAGGTTA
 TTCAATTGCTGATTCGCGCTGAGCGAGCAAATCGCGATCGCTTAAAGGAC
 AATTACAAACAGGAATCAATGCAACCGGGCAGGAACACTGCCAGCGATCAAC
 AATATTTCACCTGAATCAGGATTCTCTAATACTGGATGCTGTTCCGG
 GGATCGCAGTGGTGAATGCACTGATCATCGAGGATACGGATAAAATGCTTGAT
 GTGCGGAAGAGGCCATAATTCCGTCAGCGAGTTAGTCTGACCATCTCATCTGTA
 ACATCATTGGCAACGCTACCTTGTGCGATGTTGCAAAACACTCTGCGCATCGG
 GCTTCCCATACATCGATAGATTGTCGACCTGATTGCCGACATTATCGCGAGC
 CCATTATACCCATATAATCGCATCCATGTTGAAATTAAATCGGGCTCGAGC
 AGAGCTTCCCCTGTAATGCTCATACACCCCTTGATATTACTGTTATGTA
 GCAGACAGGTCGACAATATTGCCATTGGCATACCTGTTGATCTATATCAT
 AATATGTCATTTATGGCTCATGTCATGACCGCATGTTGACATTGATTA
 TTGACTAGTTAATAGTAATCAATTACGGGGTATTAGTTCATAGCCATATATG
 GAGTTCCGGTTACATAACTTACGGTAAATGGGCCCTGGCTGACGCCAAC
 GACCCGGCCCATGACGTAAATAAGCTGATGTTCCCATAGTAAAGCCTAG
 GGACTTCCATTGACGTCAATGGGGAGTATTACGGTAACCTGCCACTGGC
 AGTACATCAAGTGTATCATATGCCAAGTCCGCCCTTATTGACGTCAATGACGGT
 AAATGCCCGCTGGATTACGCCAGTACATGACCTTACGGGACTTCTACTT
 GGCAGTACATCTACGTTAGTCTGCTTACCATGGTGTGCGTTTGCA
 GTACACCAATGGCGTGGATAGCGGTTGACTCACGGGATTTCAGGTAACGCTC
 CCCATTGACGTCATGGGAGTTGTTGGCAGGAAATCAACGGGACTTCCAA
 AATGTCGTAATAACCCGCCCTGACGCAAATGGCGTAGGCGTGTACGGT
 GGGAGGCTATAAGCAGAGCTCGTTAGTGAACCGTCAGATGCCCTGGAGAC
 GCCATCCACGCTGTTGACCTCATAGAAGACACCGGGACCGATCCAGCCTCC
 CGGGCGGAAACGGTGCATTGGAAACGGGATTCGGCTGCCAGAGTACTCA
 CGTCCGGATCTAGCAAGCAGGTTGAGGACGCTGTTGGCTCTCTTACATG
 CAGTCAAGACTCAGGGATTGAGGACGCTGTTGGCTCTCTTACATG
 TTTGCTTGCCTCAACCTGACTATCTCCAGGTCAGGATCCAGAGTCAGGGT
 CTGATTTCCTGCTGGCTCCAGTTCAAGGAAACAGTAAACCCCTGCTCGAATA
 TTGCGCTCTCACATCTGTCATCTCCGCGAGGACTGGGGACCTGTCAGCAACAT

- continued

RAW SEQUENCE LISTING

GGCTAGCAAGGCTGTGCTGCTTGCCCTGTTGATGGCAGGCTTGGCCCTGCAGCC
 AGGCACTGCCCTGCTGCTACTCCGCAAAGCCCAGGTGAGCAACGAGGA
 CCGCTGAGGTGGAGAAACTGCACCCAGCTGGGGAGCAGTGTGAGCCGGCGCA
 TCCGCGCAGTTGGCCTCTGACCGTCATCAGCAAAGGCTGCAGCTGAACTGCG
 TGGATGACTCACAGGACTACTACGTTGGCAAAGAAGAACATCACGTCGTGACAC
 CGACTGTGCAACGCCAGCGGGGCGCATGCCCTGCAAGCCGGCTGCCGCATCC
 TTGCGCTGCTCCCTGACTCGGCCCTGCTGCTTGAGCCGGCAGCTAGGAT
 CCCAGAACCTGAACTTGTGCTGAAACTGGCAGGGATGTGAAAGCAACC
 CAGGCCCAATGCCAAGCGCGCCGCCGCGCTGGCTGTGCCCTGGGGCCT
 GGTGCTGGCGGGTGGCTCTTCTCCTCGGCTCCCTCTCGGGTGGTTAAAAA
 TCCTCAATAGGACTAATCTCAAAGCATATAATGAAAGCATTTTGAT
 GAATTGAAAAGCTGAGAACATCAAGAAGTTCTTATATAATTTACAGATACACAT
 TTAGCAGGAACAGAACAAAATTCAGCTTCAAAAGCAAATTCATCCTAGGGAA
 AGAATTGGCCTGGATTCTGTGAGCTGGCACATTATGATGTCCTGTGCTTAC
 CAATAAGACTCATCCAACTACATCTCAATAATTAAAGATGAAAGATGAAAGATT
 TCAACACATCATATTGAAACACCTCCAGGATATGAAAATGTTGGATATT
 GTACCACTTTCAGTCTTCTCCTCAAGGAATGCCAGAGGGCATCTAGTGT
 ATGTTAACTATGCAAGACTGAAGACTCTTAAATTGGAACGGGACATGAAAATC
 ATTGCTGGAAAATTGTAATTGCCAGATATGGGAAAGTTTCAGAGGAATAA
 GGTTAAAATGCCAGCTGGCAGGGGCAAAAGGAGTCATTCTACTCCGACCC
 GCTGACTACTTTGCTCTGGGTGAAGTCTATCCAGATGTTGAAATCTCCTG
 GAGGTGGTGTCCAGCGTGGAAATATCTAAATCTGAATGGTGCAGGAGACCTCT
 CACACCGAGTACCCAGCAAATGAAATGCTTATAGCGTGGAAATTGCAAGAGCT
 GTTGGTCTTCAAGTATTCTCATCCAAATGGGATCTGGGAACTATGATGCAAGAGCT
 CCTAGAAAAAATGGTGGCTCAGCACCCAGAGTACAGCTGGAGAGGAAGCT
 CAAAGTGCCTACAATGTTGGACCTGGTTACTGGAAACTTTCTACACAAAAG
 TCAAGATGCAACTTCAACTGAAAGTGAAGTGAAGAATTAAATGTTGATAGGT
 ACTCTCAGAGGGAGCTGGGAACTGGAGACAGATATGTCATTCTGGGAGGTACCCG
 GACTCATGGGTGTTGGTATTGACCCCTCAGAGTGGAGCAGCTGGTGTCTAG
 AAATTGTAAGGGAGCTTGGAAACACTGAAAAGGAAGGGTGGAGACCTAGAAGAAC
 AATTGTTGGTGAAGCTGGGATGCAAGAAGATTGGTCTCTGGTTACTGAGT
 GGGCAGAGGAGAAATTCAAGACTCCCTCAAGAGCGTGGCTGGCTTATATTAAATGC
 TGACTCATCTATAAGGAAACACTCACTCTGAGAGTTGATGTTACACCGCTGATGT
 ACAGCTGGTACACAACCTAACAAAAGAGCTGAAAGGCCATGATGAGGTTG
 AGGCAATCTCTTATGAAAGTGGACTAAAAAAAGTCCCTCCAGAGTTCACTG
 GCATGGCAGGATAAGCAAAATGGGATCTGGGAAATGATTGAGGTGTTCTCCA
 ACAGCTGGAAATGCTTCAGGCAGACGTGTTACTAAATTGGGAAACAAAC
 AAATTAGCGGCTATCCACTGTATCACGTGCTATGAAACATATGAGTGGTGG
 AAAGTTTATGATCAAATGTTAAATATCACCTCACTGTGCCCCAGGTTGGAG
 GGATGAGTGGTGTGGCCAACTCCATAGTGTCTCCCTTTGATGTTGAGATTAT
 GCTGTTAGTTAAAGAAAGTATGTCATCAGGAAATCTAGTATTCTATGAAACATCCA
 CAGGAATGAAGACATACAGTGTATCATTGATTCACTTTCTGAGTAAAGAAT
 TTACAGAAAATGCTTCAAGTCTCAGTGAGAGACTCCAGGACTTGTGACAAAGCAA
 CCACTAGTATTAAGAATGATGATCAACTCATGTTCTGGAAAGAGCATTAA
 TTGATCATTAGGGTATTACAGACAGGGCTTTTATAGGCATGTCATCTATGCTCCA
 AGCAGCCACAAAGTATGCAAGGGAGTCATCCCAAGGAATTATGATGCTCTGT
 TTGATATTGAAAGCAAGTGGACCCCTCCAAGGCCTGGGAGAAGTGAAGAGACA
 GATTATGTTGGCAGGCTTACAGTTACTGGCGAAGGCCGCTTGGAAATAAGCC
 GCTTAAGAGATCTGACCCCTAACGTTACTGGCGAAGGCCGCTTGGAAATAAGCC
 GTGTCGTTGTTCTATATGTATTTCACCATATTGCCCTTGGCAATGTG
 AGGGCCGGAAACCTGGCCCTGCTCTTGACGAGCATTCTAGGGTCTTCC
 CCTCTCCAAAGGAATGCAAGGCTGGTGAATGTCGTGAAGGAAAGCAGTTCTC
 TGGAGGCTTCTGAAGACAAACACGCTCTGAGCAGGAGACTTGGAGTGAAGTA
 CCCCCACCTGGCGCAGGTGCTCTGGGCCAAAGCCACGTGTATAAGATAC
 ACCTGCAAGGGGACAACCCAGTGCCACGTTGAGTGGAGATTGTTGGA
 AAAGAGTCAAATGGCTTCCCTCAAGCTTACAAAGGGCTGAGGAGTCCAG
 AAGGTTACCCATTGAGGTTATGGGATCTGATCTGGGGCTCGGTGACATGTTTACAT
 GTGTTTAGTCAGGGTTAAAACGTTAGGCCCCCGAACACGGGGAGCTGGT
 TTCCTTGAAAACACGATGATAATGGCAGCATGTTGGGAGGTGGAGTG
 CGAGAACGCATTCCAAACCCCTGGCAGGTGCTGTGGCTCTCGTGGCAGGGCAGT
 CTGGCGGGTGTGGTCTGGGCTCTGGGCTCACAGCTGCCACTGCA
 CAGGAACAAAGCGTGTCTGGTCTGGGCCACAGCTGTTTATCCTGAAGAC
 ACAGGCCAGGTATTCAGGTGAGCCACAGCTTCCACACCCGCTACGATATGA
 GCCCTCTGAAGAATGATTCTCAGGCCAGGTGATGACTCCAGCACCTCAT
 GCTGCTCGGCTGTCAGAGCTGGCAGGCTCACGGATGCTGAGGTGATG
 CCTGCCCAACCCGGAGCCAGCAGTGTGGGACCCCTGCTACGCCCTCAGGCTGG
 GCAGCATGAAACAGAGGAGTTCTTGACCCAAAGAAACTTCAGTGTGCA
 CCATGTTATTCCAATGACGTGTTGCGCAAGTTCACCTCAGAAGGTGACCAAG
 TTCACTGCTGTTGCTGGACGCTGGACAGGGGCAAAGAACGACCTGCTGGGTGAT
 TCTGGGGGCCACTGTTGTAATGTTGCTCAAGGATACAGTCATGGGCA
 GTGAACCATGTCAGGACACCATGTCAGGCCAACCTGCTACACCAAGGTGGTGCATTA
 CGGAAAGTGGATCAAGGACACCATGTCAGGCCAACCTGCTACAGGATCTGGGCTTA
 ACAAAACAAAAAGATGGGGTATTCCCTAAACTTCATGGGTTACGTAATTGGAAAGT
 TGGGGGACATTGCCACAAGATCATATTGTAACAAAGATCAAACACTGTTTAAAGAAA
 ACTTCCTGTAACAGGCCTATTGATGTTGAAAGTATGTCACAGGATTGTTGGCTTT
 TGGGCTTGTGCTGCCTATTACACAAATGTTGATATCCTGCCTTAATGCCCTTGTAT
 GCATGATACAGCTAACAGGCTTCTGACCTTCTGCCAACTACAGGCTTCT

- continued

RAW SEQUENCE LISTING

AAGTAAACAGTACATGAACCTTACCCGTTGGCTGGCAACGCCCTGGTCTGTGC
 CAAGTGGTGTGACGCAACCCCCTGGCTGGGCTTGCCATAGGCCATCAG
 CGCATGGTGGAACCTTGTGCTCTCGGATCCATACTGGCGAACACTCTAG
 CGCTTGTGCTCGCAGCCGGCTGGAGCAAAGCTCATAGGAACTGACAATT
 TGTGCTCTCGCGAAATATACTCGTTGATCTACGTATGATCTTCCCT
 CTGCCAAAATTATGGGACATCATGAAGCCCTTGAGCATCTGACTCTGGCTA
 ATAAGGAAATTATTTCTATGCAATAGTGTGGAATTTTGTGCTCTCACT
 CGGAAGGAAATTGTCATTAATGAATCGGCAACCGCGGGAGAGGGGGTTGC
 GTATTGGCGCTTCCGCTTCCGCTCACTGACTCGCTCGCTCGGTGTTCG
 GCTGCGCGAGCGGTACAGCTCAAAAGCGGTAAACGGTTATCCACAGA
 ATCAGGAGATAACGGCAGGAAACATGTGAGCAAAAGGCCAGAAAAGGCCAG
 GAACTGAAAGGCCCCGTTGGCTGGCTTTTCCATAGGCTCCGCCCCCTGA
 CGAGCATCACAAAATGACGCTCAAGTCAGGGTGCAGAACCCGACAGGACT
 ATAAGAGATACAGGCGTTCCCGATACTGTGCGCTTCTCCCTGGGAGGCGTGGCG
 ACCCTCGCGTACCGGATACTGTGCGCTTCTCCCTGGGAGGCGTGGCG
 CTTCTCATAGCTCAGCTGTAGGTATCTCAGTGTGGTGTAGGTGCTGCTCCA
 AGCTGGGCTGTGCAAGAACCCCGTTAGCCGACCGCTGCGCTTATCCG
 GTAATATCGCTTGAGTCAACCCGTAAGACACGACTTATGCCACTGGCAGC
 AGCCATGGTAACAGGATTAGCAGACGGAGGTATGTAGGCGGTGCTACAGAGTT
 CTGAAGTGGTGGCCTAACTACGGCTACACTGAAGAACAGTATTGGTATCTGC
 GCTCTGCTGAAGCAGTAACTTCGAAAGAAGGTGGTAGCTTGATCGGCA
 AACAAACACCCTGGTAGCGGGTTTTTGTGCAAGCAGCAGATTACGCG
 CAGAAAAAAAGATCTCAAGAAGATCTTTGATCTTTCTACGGGGTCTGACGCTC
 AGTGGAAAGAAAACACGGTAAAGGAGTTTGTGATGAGATTATCAAAAAGGAT
 TTCACTTAGCTTTAAATTAAGGTTTAAATCAATCTAAAGTATATATAG
 AGTAAACTTGGCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCAGC
 GATCTGCTATTGCTCATCAGTGTGCTGACTC

SEQ ID NO: 37. NUCLEOTIDE SEQUENCE OF PSHUTTLE IRES
 CATCATCAATAATACCTTATTTGGATTGAAGCCAATATGATAATGAGGGGGTG
 GAGTTTGTGACTGGCGGGCGTGGGAACGGGGGGGTGACGTAGTAGTTGT
 GGCGGAAGTGTGATGTGCAAGTGTGCGGAAACATGTAAGCGACGGATGTTG
 CAAAAGTGACGTTTTGGTGTGCGCGGTGTAACAGGAAGTGAATTTCGCG
 CGGTTTAGGCGGATGTTGTAGTAAATTGGCGTAACCGAGTAAGATTTGGCA
 TTTTGGGGAAACACTGAATAAGAGGAAGTGAATCTGAATAATTGGTGTACTC
 ATAGCGCTGTAATCTGAATAATGCAATTACGGCTTACAGGCTTATCTGACGCT
 ATATGGAGTTGGCGTITACATAACTTACGGTAATGGCCCTGTGCGTACGCC
 CAACGACCCCGCCATTGACGCTAATAATGACGTATGTTCCATAGTAACGCC
 ATAGGAGCTTCCATTGACGTCAATGGGTGAGTATTACGGTAAACTGCCACT
 TGGCAGTACATGGCGTGTAGCGTTGACTCACGGGATTCTCAAGTCTC
 CGGTAATGGCCGCTGGCATATGCCAAGTACGGCTTATGGACTTCT
 ACTTGGCAGTACATCTACGTTAGTCATGCTTACCATGGTATGCGTTTTG
 GCAGTACATGGCGTGTAGCGTTGACTCACGGGATTCTCAAGTCTC
 CACCCATTGACGTAATGGGAGTTTGGCAACCAAATCAACGGGACTTTC
 CAAATGCTGTAACACTCCGCCATTGACGCAATGGGCGTAGGCGTGTAC
 GTGGGAGGTATATAAGCAGAGCTGGTTAGTGAACCGTCAGTCCGCTAGA
 GATCCACCATGGCTAGCGGTGCCGACGTGCCCCCTGCTGGCAGCCCTTC
 TCAAGGACCCGCATCTACATTCAAGAACAGTGGCCTTCTGGAGGGCTGCG
 CTGGCCTGGGGATGGCCGAGCTGGCTTCTGCTTACACTGCCACTGAGA
 ACGAGCCAGACTGGCCAGTGGTTCTCTGCTTAAGGAGCTGGAGGTGG
 AGCCAGATGACGACCCATAGGAAACATAAAAGCATTGCTGGTTGCGCTT
 CCTTCTGTAAGAAGCAGTTGAAGAATTACCCCTGGTGAATTGGAAACTGG
 ACAGAGAAAGAGCGAAACAAATTGCAAGAACACAAACATAAGAAGAAGA
 ATTGAGGAAACTGCGGAGAACGTGGCGTGGCATCGAGCAGCTGCGTCC
 GGATTAGAGATGACCCCTAACGTTACTGGCGAAGCCGTTGGAATAAGGC
 CGGTGCGTGTGTATGTTCCACCATATTGCGCTTGGCAATGTC
 GAGGGGGCGGAAACCTGGCCCTGCTTCTGACGAGCATTCTAGGGCTTTC
 CCTCTGCAAAAGGAATGCAAGGTCTGGTGAATGCTGTGAAGGAAGGAGTCC
 CTGGAAGCTTCTGAAAGACAAACAGCTGTAGCGACCCCTTGCAAGCGGAA
 ACCCCCCACCTGGCAGGGCTGGCCAAACAGCTGTATAAGATA
 CACCTGCAAAGGGCGCACAAACCCAGTGCCACGGTGTGAGTTGGATAGTTGG
 AAAGAGTCAAATGGCTCTCTCAAGGTATTCAACAGGGCTGAGGAGTCC
 GAAGGTACCCATTGTGAGGACTGATCTGGGCTCGGTGCACTGCTTACA
 TGTTGTTAGTCAGGTTAAAAAACGCTTAGGCCCCCGAACACGGGAGCTGG
 TTCTCTGAAAACACGATAATGGCGGCGCTGAGCTAACGCTTACGATA
 AGATATGCGATCAGGATCTAGATAACTGATCATAAATGCCATACCAATTG
 TAGAGGTTTACTTGTGTTAAAAACCTCCACACCTCCCTGAAACCTGAA
 AAAATGAATGCAATTGTTGTTAACTTGTGTTATGCAAGCTTAAATGGTAC
 TAAAGCAATGACATACAAATTCAACAAAGCATTGTTACTGATCTGACT
 TGTTGTTGCTCAAACGATACTGATCTTAACGCGGATCTGGCGTGTTAAG
 GGTGGGAAAGAATATAAGGTTGGGGCTTATGTTGATCTGTTGAGCT
 CAGCCGCGCCCATGAGCACCAACTCGTTGATGAGCATTGAGCTCAT
 ATTGACAAACGCGCATGCCCATGGCGGGGTGCGTCAGAATGATGGGCT
 CGAGCATTGATGTCGCCCCCTGCCCCGAAACTCTACTACCTGACCTACGA
 GACCGTGTCTGAAACGCCGTTGGAGACTGCGACCCCTCGCCGCGCTTCAGCG
 CTGCAAGCACCAGCCGCGGGATGTTGACTGACTGACTTGTGCTTCTGAG
 CAAGCAGTGCAGCTTCCGTTATCCGCCCCGATGACAAGTGTGAGGCTTTT

- continued

RAW SEQUENCE LISTING

GGCACAAATTGGATTCTTGACCCGGAACTTAATGCTGTTTCAGCAGCTGGT
 GACTCGGCCAGGGTTCTGCCCTGAAGGCCTCCCTCCCAATGCGTTT
 AAAACATAAATAAAAAAACAGACTCTGTTGATTTGGATCAAGCAAGTGTCTTG
 TGTCTTATTAGGGGTTTGGCGCGCGTAGGCCCCGGGACAGCGGCTCGG
 TCGTTAGGGCTGCTGTGATTTCAGGACGTGTAAGGTGACTCTGGATGT
 TCAGATACATGGGCATAAGCCGTCTGGGGTGGAGGTAGCACCACTGCAGAG
 CTTCATGGGGGGTGTGAGTATCAGTCAGTCAGCAGGAGCGCTGGG
 CGTGGCTGCTTAAAGCGGTTAACGCTGGGATGGGTGATCTGGGATAT
 GAGATGCATCTGGACTGTATTAGGGCTATGTTCCAGCCATATCCCTCC
 GGGGATCATGGTGTGAGGAAACCCACAGCAGTGTATCCGGTGCACTTGGGAA
 ATTGTCATGAGCTTAAAGGAAATTCGCTGGAGAACATTGGAGACGCCCTTG
 ACCTCAAGATTTCATGCTTCATAATGATGGAATGGGCCACGGCG
 GCGGCTGGCGAAGATATTCTGGGATCACTAACGTCTAGTTGTTCCAGGA
 TGAGATGCTCATAGGCCATTAAAGGGGGGGAGGGTGCAGACTGCG
 GTATAATGGTCCATGGGCCAGGGCGTAGGTTACCCCTCACAGATTGCTATT
 CCACGGCTTGGACTCATGGGGGATCATGCTCACCTGGGGCATGAGAAA
 AACGGTTCCGGGGTAGGGAGATCAGCTGGAGAACAGGGTCTGAGCAG
 CTGCGACTTACCGCAGGGTGGGCCAAATCACACCTTACGGCTGCAA
 CTGGTAGTTAAAGAGAGTCGAGCTGGCTCATCCCTGAGCAGGGGGCACTC
 GTAAAGCATGCTCTGACTCGCATGTTCCCTGACAAATCCGGAGAAGCG
 TCGCCGCCAGCGATAGCAGTTCTGCAAGGAAGCAAAGTTTCAACGGTTGA
 GACCCTCGGGCTAGGCATGCTTTGAGCAAGCAGGTTCCAGGGGT
 CCCACAGCTGGCTCACCTGCTACGGCATCTCGATCCAGCATATCTCTCGTT
 CGCGGGTTGGGGCGGTTTGGTAGGGCAGTGTGGCTCTGGCAGAC
 GGCCAGGGTATGTCTTCCACGGGCGAGGGCTCGTCAGCGTAGTCTGGG
 CACGGTAGGGGTGGCTCCGGGCTGGCGCTGGCCAGGGTGGCTTAGGG
 TGGCTCTGCTGGCTGAAGGGCTGGGGCTTGGCGCTGGCCAGGT
 AGCATTGACCATGGTGTCAAGTCCAGGCCCCTCCCGGGCTGGCC
 GCAGCTTGGCCCTGGAGGGCGCCACGAGGGGAGTGCAGACTTTGAGG
 GGTAGAGCTTGGGGCGAGAAAATACCGATTCCGGGAGTAGGCATCCGCC
 GCAGGGCCCGAGCGGTGCACTTCCACGGGAGGTGAGCTCTGGCGT
 GGGGTCAAAACAGTTTCCCCATGCTTTGATGCGTTTACCTCTGGTT
 CCATGAGCCGGTGTGACGCTCGGTGACGAAAGGCTGTCGGTGTCCCGTATA
 CAGACTTGGAGGGAGTTAAACGAAATTCAATAGCTGTTGATGGCGGGATA
 TAAAAGCAAGGCTGCTCAAAAATCAGGCAAAGGCTCGCGAAAAAGAAAAG
 CACATGCTAGTCATGCTCATGAGATAAGGCAGGTAGCTCCGAACACACA
 GAAAAGACACCAATTCTCTAAACATGCTGCGGTTCTGATAAACACAAA
 ATAAAATAACAAAAAAACATTAAACATTAGAAGGCTGCTTACACAGGAAAACA
 ACCCTTAAAGCATAAGACGGACTACGGCCATGCGCGTGACCGTAAAAAAACT
 GGTACCGTGTATAAAAGCACCACCGACGCTCTGGTATGTCGGAGTCAT
 ATATGTAAGACTCGGTAACACATCAGGTGATTCACATCGGTAGTGTAAAAAG
 CGACCGAAATAGCCCGGGGAAATACATACCCGCAAGCGTAGAGAACACATTACA
 GCCCCATAGGGTATAACAAAATTAGGAGAGAAAAAACACATAAACACTG
 AAAAACCCCTCTGCTAGGGAAAATAGCACCTCCCGCTCCAGAACACATACAG
 CGCTTCCACAGGGCAGCCATAACAGTCAGCTTACAGTAAAAAGAAAACCTA
 TAAAAAAACACCACTCGACACGGCACAGCTCAATCAGTCAGTGTAAAAAG
 GCGCAAGTGCAGAGGGTATATAGGACTAAAAAATGAGCTAACGGTTAAAGT
 CCACAAAAACACCAAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
 GAAAACCCACAACCTCTCAATGTCATCCCAACATACAAGTTACTCCGCCCC
 TAAGTCACCCGCCCTCCACGGCCCGCCACGGTCACAAACTCCACCCCCCT
 CATTATCATATTGGCTTAATCAAAATAAGGTATATTGATGATGTTAAATTAC
 ATGCACTGGATCATATGCGGTGTGAAATACCGCACAGATGGTAAGGAGAAAATA
 CCGCATCAGCGCTTCCGCTTCGCTCACTGACTCGCTCGCTCGGT
 TCGGCTGCGGGAGCGGTATCAGCTCACTAACGGGTAATACGGTTATCCAC
 AGAATCAGGGGATAACCGAGAAAAGACATGAGCAGGAAAAGGCCAGCAGG
 CAGGAACCGTAAAAGGGCGGGTTGGCTGGCTTTCCATAGGCTCGCCCC
 TGACGAGCATCAAAAAATCAGCGCTCAAGTCAGAGGTGGGAAACCGACAGG
 ACTATAAGAGCATCGGGTGTGACGGGTTCCCGCTGGGCTCTCGT
 CCGACCCCTGGCGCTTACCGGATACCTGGCTCGGCTTCCCTGGGAGCGTG
 GCGCTTCTCATAGTCACGCTGTAGGTATCTAGTTCGGTGTAGGTGCTCGCT
 CCAAGCTGGGCTGTGTCACGAAACCCCGTTAGCCGACGGCTCGCCCTAT
 CGGTAACTATGCTGTGAGTCAGGGTAAGCACGACTTATGCCACTGGC
 AGCAGCAGCTGTAACAGGATTAGCAGAGGGTGTAGGGCTGCTACAGA
 GTCTTGAAGTGGCTCAACTACGGCTACAGTAAAGGGAGTATTGGTATC
 TCGCTCTGCTGAGGCCAGTTACCTGGAAAAGAGTTGGTAGCTCTGATCCG
 GCAAACAAACACCGCTGGTAGGGGTTTTTGTGCAAGCAGCAGATAC
 GGGCAGAAAAAAAGGATCTAAGAAGATCCTTGTATCTTCTACGGGCTGAC
 GCTCACTGGAGGAAAGAAACTCACGTTAAGGGATTGGTATGAGGATTACAAA
 GGATCTTCACTTAGATCCTTTAAATTAAAGGTTGGTATGAGGATTACAAA
 TATATGAGTAACATTGGTCTGACAGTTACCAATGCTTAATCAGTGGAGGACCTATC
 TCAGCGATCTGCTATTGCTCATCCATGTCAGTGGCTGACTCCCGCTGAG
 AACTACGATACGGAGGGCTTACCATCTGGCCCACTGCTGCAATGATACCGG
 AGACCCACGCTACCGGCTCCAGATTATCAGCAATAAACAGCCAGCCGAAG
 GCGCAGCGAGAAGTGGCTGCAACTTATCCGCTCCATCCAGTCTATTAAAT
 GTTGGCGGGAAAGCTAGAGTAAGTAGTTCGCGAGTTAAGTAGTTGCGAACGTTG

- continued

RAW SEQUENCE LISTING

TTGCCATTGCTGCAGCCATGAGATTATCAAAAGGATCTTCACCTAGATCCTTT
 ACGTAGAAGCCAGTCGCAGAACACGGTGTGACCCGGATGAATGTCACTAC
 TGGGCTATCTGGCACAGGGAAAAGCGAACAGGCAAAGAGAAAGCAGGTAGCTTG
 AGTGGGCTTACATGGCGATAGCTAGACTGGGGGGTTTATGGACAGCAAGCGAA
 CGGAAATTGCCAGCTGGGGCCCTCTGGTAAGGTTGGGAACGCCCTGCAAGATA
 AACTGGATGGCTTCTTGCAGCAAGGATCTGATGGCGCAGGGGATCAAGCTCT
 GATCAAGAGACAGGATGGGGATCTGGCATGATTGAACAAGATGGATTCAGCAG
 CAGGTTCTCGGGCGCTGGGGAGAGGGCTATTCGGCTATGACTGGGACAAAC
 AGACAACTGGCTGCTGTGATGCCCGTGTCCGGTGTGCAAGGGCAGGGCG
 CGGGTTCTTTGTCAAGACCGACCTGTCCGGTGTGCAAGTAAGTCAAGACG
 AGGCAGGAACTGGGCGTGGGCAAGCAGCTGGGCTTCTGGCCAGGCTGTG
 CTGACCTTGTCACTAGCGGAAAGGCACTGGCTATGGGCAAGTCCG
 GGGCAGGATCTCTGTATCACCTTGCTCTGGCGAGAAAGTATCCATCATGG
 CTGATGCAATGCCGGCTGTGATACGTTGATCCGGTACCTGCCATTGACC
 ACCAAGGGAAACATGCCATCGAGCGACACTCGGATGGAAAGCCGGCTTG
 TCAGTCAAGGATCTGGACAGAGACATCGGGGCTCGGCCAGCGAACTGT
 TCGCAAGGCTCAAGGGAGCATGCCGACGGCAGGGATCTGTCGTGACCCAT
 GGCAGTGCCTGCTGGCAATATCATGGTGGAAATGGCCGTTTCTGGATTCA
 TCGACTTGTGGGGCTGGGTGCGGACCCCTATCAGGACATAGGGTTGGCTA
 CCCGTGATATTGCTGAAGAGCTGGGGCGAATGGGCTGACCGCTTCTCGTG
 TTACGGTATGCCGCTCCGATTCCGAGCCATGCCCTTATGCCCTTGTGA
 CGAGTTCTCTGAATTGGTAAATTCAGCTATTTTAAATA
 GCGGAATCCGACCATCCCTTATAAATCAAAGAATAGACCGAGATAAGGTTG
 AGTGTGTTCCAGTGGGAACAGACTGGCAACTTAAAGAACGTGGACTCCAACG
 TCAAAGGGCAAAACCGTCTATCAGGGCAGGCCACTACGTGAAACCATCAC
 CCTAATCAAGTTTTGTGGCTGAGGTGCGTAAAGCACTAAATCGGAACCCAAA
 GGGAGCCCCGATTAGAGCTGACGGGAAAGCCGCAACGTTGGCAGAAA
 GGAAGGGAAAGGAAAGCAGGGGCTAGGGCCTGGCAAGTGTAGCG
 TCACTGCGCTAACCAACACCCCGCGCTTAATGCGCCGCTACAGGGCG
 GTCCATTGCCATTCAAGGATCGAATTAAATTCTTAATTAA

SEQ ID NO: 38. Amino acid sequence of Her-2 antigen:
 MASELAAALCRWGLLALLPPGAASTQVCTGTDMLKLRPLPSPETHLDMLRHLYQGCQ
 VVQNLLETLYLPNTNASLFLQD1QEVQGYVLIAHNQRQVPLQLRLIVRGTQLFEDNY
 ALAVLVDNGDPLDSVAPAAAGATPGGLQELQLRSLTEILKGGVLIRRSPQLCHQDTVLWE
 DVFRKNNQNLALVMDTNRSRACHPCAPMCKANHCWGESSQDCQTLTRTICTSACAR
 CKAPLTDCCHEQCAAGTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTEFESMP
 NPEGRYTFGASCVTACPYNLSTDVGSTLVCPLHNQEVTAEDEGTQRCEKSKPCA
 RVCYGLGMELRLEARAITSANVQDFVGCKKIFGSLAFLPESFDGPASGTAPLOPEQ
 LQVFETLEEITQVNSAWPDSPNLSVFPQNLRVIRGRILHNNGAYSLTLQGLGISWLGL
 RSLQELGSGLALVHRNARLCFVHTVPWDQLERNPQHQLLHSGNRPEEDCVGEFGVC
 YSLCAHGHCHWPGPQTCVNCSHFLRGQECVEECRVLQGLPREYVNARHCLPCHPE
 CQPQNGSVTCFCPEADQCVACAHYKDPPFCVARCPSGVKPDLISYMPIWKFPDEEG
 ACQPCPINCTHSVLDKGCPAEQRASPLTSIIISAVVGLLLVVVLGVVRGILIKRRQQK
 IRKYTMRRRNEDLGPSSPMDSFYRSLEDEDMGELVDAEYLVPPQGFPCDPPTPGT
 GSTAHRRHRSSSARNGGDILTGMEPSEGGPRS PRAPSEGTGSDVFDGLALAVGV
 TKGLQSLSPQDLSPLQRYSEDPTLPLPSETDGKVAPLSCSPQPEVNQSDVQPKSPL
 TPEGPPSPARPTGATLERAKTSLPGKNGVVKDVFTEGGAVENPEFLAPREGTASPPH
 PSAPFSPAFDNLFFWDQNSSEQGPPPSNFECTPTAENPEFLGLDVPV
 (signal sequence underlined)

SEQ ID NO: 39. Nucleic acid sequence encoding the Her-2 antigen amino acid

sequence of SEQ ID NO: 38
 ATGGCTAGCGAGCTGGCGCCCTGTGTAGATGGGACTGCTGCTGGCTCTGCTG
 CCTCTGGAGGCCCTTACACAGGTCTGCAACGGCACCGACATGAAGCTGAGA
 CTGGCCCGAGGGAGACACACTGGGACATGCTGGGGCACCTGTGCGGACCTGG
 CTGGCAAGGTGGTCCAGGGAACTCTGAAACTGACCTACCTGCCACCAAGCCAG
 CCTGAGCTTCTGCAGGACATCAGGAAGTGCAGGGTACGTCCTGATGCCCA
 CAACCAAGGTGGCCAGGTGCCCCCTGAGGGCTGAGAATCTGGGGGGACCC
 AGCTGGAGGACAATACGGCCCTGGGGCTGGGACACGGGGACCCCTCTGG
 ATAGCTGGCCCCCTGCTGCTGGGGTACACCTGGGGACTGCAAGGAACTGCA
 CTGGGGAGCTGACCGAGATCTGAAGGGGGGGCTGCTGATCAGGGGAGGCC
 TCAGCTGTGCCACCAAGGACACGGTGTGGGAGGACGTGTTCCGAAGAACAA
 CCAGCTGGCCCCCTGTGTAGATGGACACCAAAGAAGCCGGGCTGCCACCCCTG
 CGCCCCCATGTGCAAGGCAATCTGCAACAGCGCTGGGGCAGATGCAAGGCCCC
 AGACCTTGACCCGGACCATCTGCAACAGCGCTGGGGCAGATGCAAGGCCCC
 CTGCCTACCGACTGTCGCCACAGTGCGCCGCTGGCTGCACCGGCCCCAA
 GCACAGGGATTGCCCTGGCCTGCTGCACTTCAACACAGCGCATTGCGAGCT
 GCACTGCCCTGCCCTGTGTGACATACAACACCGACACCTTGAGGAGCATGCCAA
 CCCCAGGGCCGGTACACCTTGGCGCCAGCTGCAACCTGGTGTGACCGCCTGCCCC
 CTACCTGAGCACCGACGGTGGGCTGGGAGAGATGCGAGAAGTGCAGCAAGCCT
 GGGCAAGAGTGTGCTACGGCTGGGAGGACATGGAAACACCTGAGAGAGGCCAGGCC
 ATCACCAAGCGCCAACGTGCAGGACTTCGTGGCTGCAAGAAGATTTCGGCTCC
 CTGGCTTCTGCCGAGAGCTCGACGGCAGTCCTGCCCTGGCACCGCCCC
 CTGCGAGCTGAGCAAGTCAAGGATGGGAGGAGATGCGAGAAGTGCAGCAAGCCT

- continued

RAW SEQUENCE LISTING

CTGTACATCAGGCCCTGGCCCACAGCTTCCCAACCTGAGCGTGTCCAGAAC
 CTGAGAGTGATCGGGGGCAGAACATCTGCACAAACGGCCCTACAGCCTGACCCCTG
 CAGGGCCCTGGGAATCAGCTGGCTGGGGCTGGCGAGCCTGAGGAACCTGGGATC
 TGGCCTGGCTCTGGTCACCGGAACGCCCGCTGCTTCGTCACACCGTGC
 CTGGGACAGCTGTTAGAAAACCCCCACCAAGCTCTGTCACAGCGGCAACCG
 GCCCGAAGAGGGATTGCGTGGGGCAGGGCTTCGTCGACTACTCCCTGCGCCCA
 CGGCCACTGTGGGGACCTGGCCCTACCCAGTGCCTGAGACTGCAGCCACTTCT
 CGGGGGGAAAGAATGCGTGGGAAGATGCGGGGTGCTGCAGGGACTGCCCCGG
 AATACTGTAACCCAGACACTGCCTGCCTTGCACCCCCAGTGCAGCCAGA
 ATGGCAGCGTACCTGCTCGGACCCGAGGCCATCGAGTGTGGCTGCGCC
 CACTAAAGGCCACCCATTCTGGCTGGCCAGATGCCCAAGCGGCCCTGAAGCCC
 GACCTGAGCTACATGCCCATCTGGAGTTCCCCGACAGGAAAGGGCCCTGCCAG
 CCTTGGCCCATACTGCACCCACAGCTGGCTGGACTGGACGACAAGGGCTGC
 CCTGCGAGCAGAGAGCAGCCCCCTGACCAGCATCATCAGGCCGTGGTGG
 ATGGCTGGGGTGGGTGGTGGTGGCTGGGATCTGGCATCTGATCAAGCGGCC
 GCAGCAGAAAGATCCGGAAGTACACCATGCGGGGAACGAGGACCTGGGCCCC
 CTAGCCCCATGGACAGCACCTCTCACCGGCTCTGAGGAGATGAGGACATGG
 GCGAGCTGGTGACGCCAGGAATACTGGTGCCTCAGCAGGGCTTCTTCTGCC
 CGAACCTTACCCCTGGCACCGCCTACCGCCACAGACGCCACAGAACAGCA
 GCGCAGAAACGGCGGAGGCGACCTGACCCCTGGGAATGGAACTAGCGGGAG
 GGACCTCCCAGAGCCCTAGAGCCCTAGCGAGGGCACCGCAGCAGTGT
 CGATGGCGATCTGGCGTGGCGTGACCAAGGGACTGCAGAGGCTGAGCCCC
 AGGACCTGTCCTGGCAGAGATAACAGCAGGAGACCCACCCCTGCCCTGCCCA
 GCGAGACAGATGGCAAGGTGGCTGGCTGGCAGCTTGACGCCCTCAGCCCGAGTT
 GTGAACCCAGAGGGACGTGCAAGCTTGGCAGACACCCGAGGGACCTCCA
 AGCCCTGCCAGACCTACCGGCCACCCCTGGAAAGAGCCAAGACCTGAGCCC
 CGGCAAGAACGGCGTGGTGAAGAGCTTGGCACCTTGGAGGGCGCGTGGAAAA
 CCCCGAGTCTCTCCCCGCTTCGACAACCTGTTCTGGGACAGAGCAGCGA
 CAGCCTCTCCCCGCTTCGACAACCTGTTCTGGGACAGAGCAGCGA
 GCAGGGCCCACCCCCCAGCAATTGAGGGACCCCCACCGCCGAGAATCTGA
 GTTCCTGGGCTGGACGTGCGCGTGTGA

SEQ ID NO: 40. Amino acid sequence of heavy chain of the anti-CD40 antibody
 CP870, 893:

MDWTWRLFLVAAATGAHSQVLVQSGAEVKPGAVKSCKASGYFTGYMMHW
 VRQAPQGLEWMGWINPDSGGTNYAQKFQGRVTMTRDTSIAYMELNRLRSDDT
 AVYYCARQPLQYCTNGVCSFYDWFQGTLTVSSASTKGPSVFLAPCSRSTS
 TAALGCLLVKDYFPEPVTVWSNSGALTSGVHTFPALQSSGLYLSLSSVTPSSNFGT
 QTYTNCNDHKPNTKVDKTVERKCCVECPCCPAPPVAGPSVFLFPKPDKTLMSRT
 PEVTCVVVDVSHEDPEVQFNWYVGVEVHNAKTKPREEQFNSTFRVSVLTVH
 WLNGKEYKCKVSNKGLPAPIEKTISKTKQGPREPQVYTLPPSREEMTKNQVSLTCLV
 KGFYPSDIAVEWESENQOPENNYKTTPPMLDSGSSFLYSKLTVDKSRWQQGNVF
 SVMHEALHNHYTQKSLSLSPGK.

SEQ ID NO: 41. Acid sequence of the light chain of the anti-CD40 antibody
 CP870, 893:

MRLPAQLLGLLNLWFPGRSRCDIQMTQSPSSVVASVGDRVTITCRASQGIYSLWAWYQ
 QKPGKAPNLIIYIYSTLQSGVPSRFSRGSGSGTDFTLTISSLQPEDFATYYCQQANIFPL
 TPGGGTKVEIKRTVAAPSIFIFFFFSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNAL
 QSGNSQESVTEQDSKDSTYLSLSSLTLSKADYEHKVYACEVTHQGLSSPVTKSFNR
 GEC.

SEQ ID NO: 42. Acid sequence of the heavy chain of the anti-CTLA-4 antibody
 Tremelimumab

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIYWDG
 SNKYYADSVKGRFTISRDNSKNLTYLQMNSLRAEDTAVYYCARDPRGATLYYYYGM
 DVWGQTTVTVSASTKGPSVFLAPCSRSTS ESTAALGLVVDYFPEPVTVWSNS
 GALTSQVHTFPALQSSGLYLSLSSVTPSSNFGTQTYTCNVDHKPSNTKVDTKVER
 KCCVECPCCPAPPVAGPSVFLFPKPDKTLMSRTPEVTCVVVDVSHEDPEVQFNWY
 VDGVEVHNAKTKPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
 ISKTKQGPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESENQOPENNYK
 TPPMLDSGSSFLYSKLTVDKSRWQQGNVFCSVMEALHNHYTQKSLSLSPGK

SEQ ID NO: 43. Acid sequence of the light chain of the anti-CTLA-4 antibody
 Tremelimumab

DIQMTQSPSSLSASVGDRVTITCRASQSIINSYLDWYQQKPGKAPKLIYAASSLQSGV
 PSRFSGSGSGTDFTLTISSLQPEDFATYYCQYystPFTFGPGTKVEIKRTVAAPSFI
 FPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
 SLSSTLTLSKADYEHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 44. Nucleotide sequence of CpG 7909
 5' TCGTCGTTTGTGCGTTTGTGTT3'

SEQ ID NO: 45. Nucleotide sequence of CpG 24555
 5' TCGTCGTTTTCGGTGCTTT'

-continued

RAW SEQUENCE LISTING

SEQ ID NO: 46. Nucleotide sequence of CpG 10103
5' TCGTCGTTTCGGTCGTTT3'

SEQ ID NO: 47. Amino acid sequence of eGFP
MSVKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVP
WPLTVTTLTYGVQCFCSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEV
KPEGDTLVNRKIGDIFKEDGNILGHKLEYNNYNHSNVYIMADQKNGIKVNFKIRHNIE
DGSVQLADHYQQNTPIGDGPVLPPDNHLYSTQSALSKDPEKRDHMVLLFVTAAGI
TLGMDELYK

SEQ ID NO: 48. Amino acid sequence of HBV core antigen
MDIDPYKEFGATVELLSFLPSDFPSPVRDLDDTASALYREALESPHECSPHHTALRQAI
LCWGELMTLATWVGNNLEDPASRDLVVNNYVNTNMGLKIRQLLWFHISCLTFGRETQL
EYLVSFGVWIRTPPAYRPPNAPILSTLPETTVRRRGGRSPRRRTSPRRRSQSP
RERRRSQSRESQC

SEQ ID NO: 49. Amino acid sequence of HBV surface antigen
MENITSGFLGPLVVLQAGFFFLTRILTIPQSLDSWWTSLNFLGGSPVCLGQNSQSPTS
NHSPPTCCPPICPGYRMCLRFIIIFLFLVLLDYQGMLPVCPFLIPGSTTSTG
PCKTCTPAQGSNSMFPSCCCTKPTDGNCTCIPIPSSWAFAKYLWEASVRFWSLSSL
VFPVQWVGLSPTVWLSAIWMMWYWPSPSYIVSPFIPLLLPIFFCLWVYI

SEQ ID NO: 50. Amino acid sequence of Rhesus PSMA ECD protein:
MASETDTLLLWVLLWVPGSTGDAAHHHHHHHKSSEATNI TPKHNMKAFLDELKAENI
KKFLHNFQTQIPHLAGTEQNQFLAKQIQSQWKEFGLDSVELTHYDVLLSYPNKTTHPNYI
SIINEDGNEIFNTSLFEPFPAGYENVDIVPPFSAFSPQGMPEGDLVYVNYARTEDFFK
LERDMKINCNSGKIVIARYGKVRGNKVNAQLAGATGVILYSDPADYFAPGVKSYPDG
WNLPGGVQRGNINLNQAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVPHIGYDA
QKLLKEMGGGSAPDSSWRGSLKVPYNVGPFTGNSTQKVKMHIHSTSEVTRIYNNVI
GTLRGAVEPDRYVILGGHRDSWVFGIDPQSGAAVHVIEIVSFGTLKKEGWRPRRTI
LPASWDAAEFGLLGSTEWAEEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSL
VYNLTKEASDPEFGEGKSLYESWTKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIAS
GRARYTKNWETNKFSSPLYHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELA
NSVVLPDFCDRDYAVVLRKYADK1YNNISMKHPQEMKTYSVSFDSLFSAVKNFTEIAS
ERLRDFDKSNILLRMMNDQLMFLERAFIDPLGLDRPFYRHVIYAPSSHNKYAGESF
PGIYDALFDIESKVDPDPSQAWGEVKRQISIATFTVQAAAETLSEVA

SEQ ID NO: 51. Amino acid sequence of rat Her-2 p66 peptide (H-2d T cell epitope)
TYVPANASL

SEQ ID NO: 52. Amino acid sequence of rat Her-2 p169 peptide (H-2d T cell epitope)
DMVLWKDVFRKNNQL

SEQ ID NO: 53. Amino acid sequence of HBV core antigen p87 peptide
SYVNTNMGL

SEQ ID NO: 54. Amino acid sequence of a Rat Her-2 Antigen (rHer-2):
MASELAAWCRWGFLLALLPPIAGTQVCTGTDMLKRLPASPETHLDMLRLHYQGCQ
VVQGNLELTYVPANASLFLQDQIPEVQGYMLIAHNQVKRVLQRLRIVRGTQLFEDKY
ALAVLDNRDPQDNVAASTPGRTPEGLRELQRLRSLTEILKGGLVIRGNPQLCYQDMVL
WKDVFRRKNNQLAPVTDNTNSRACPPCAPACKDNHCVGESPEDCQILTGTCTS
ARCKGRLPTDCCHQCAAGCTGPKHSDCLACLHFHNHSGICELHCPALTVYNTDTFES
MHNPEGRYTFGASCVTTCPYNYLSTEVGSTLVCPPNNQEVTAEDGTQRCEKCSKP
CARVYQGLMGMLHRLGARAITSNDVQEFDGCKKIFGSLAFLPESFDGDPSSCIAPLPE
QLQVFETLEEITGYLYIISAWPDSLRLSVFQNLRIIRGRILHDGAYSLTLQGLGIHSLGL
RSLRELGSGLALIHRNAHLCPVHTVFWQDFLFRNPHQALLHSGNRFEEDCGLLEGLVCN
SLCAHGHCWGPGBTQCVNCSSHFLRGQECVEECRVWKGKPREYVSDKRCLCPHPEC
QPNNSETCFQGEADOCACAAHYKDSSSCVARCPGKVQPDLSYMPIWKYDPEEGIC
QPCPINCTHSCVLDLDERCPAEOQRASPVTIATVVGULLFLILVVVVGILIKRRRQKIRK
YTMRRNEDLGPSPMGSTFYRSLLLEDDDMGDLVDABEYLVPQQGFPDPTPGTG
TAHRRHRSSTSRRGGELTLGLEPSEEGPPRSLPAPSEGASDVFDFGDLAMGVTKG
LQLSLSPHDLSPLQRYSEDPTLPLPPETDGYVAPLACSPQPEFVNQSEVQQPPLTPE
GPLPPVRPAGATLERPKTLSPGKNGVVKDVFAFGGAVENPEFLVPREGTASPPHPSP
AFSPAFCNDNLFFWDQNSSEQGPPPSNFEGTPTAEENPEFLGLDVPV

SEQ ID NO: 55. Amino Acid Sequence of Rhesus PSMA antigen:
MASARRPRWLCAAGALVLAGGFLLGFLFGWFIKSSSEATNITPKHNMKAFLDELKAENIK
KKFLHNFQTQIPHLAGTEQNQFLAKQIQSQWKEFGLDSVELTHYDVLLSYPNKTTHPNYISII
NEDGNEIFNTSLFEPFPAGYENVDIVPPFSAFSPQGMPEGDLVYVNYARTEDFFKLERD
MKINCNSGKIVIARYGKVRGNKVNAQLAGATGVILYSDPADYFAPGVKSYPDGWNLPGG
GVQRGNILNLNQAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVPHIGYDAQKLLEKMG
SASPDSWRGSLKVPYNVGPFTGNSTQKVKMHIHSTSEVTRIYNNVIGTLRGAVEPDY
VILGGHRDSWVFGGIDPQSGAAVHVIEIVSFGTLKKEGWRPRRTIIFASWDAEEFGLLG
TEWAEEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLTVYNLTKELESPEFEGK
SLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGRARYTKNWETNKFSSYPL

-continued

RAW SEQUENCE LISTING

YHSVYETYELVEKFYDPMFKYHLTVAQVRGGMVFELANSVVLPMFCDR DYAVVLRKYADKI
 YNISMKHPQEMKTYSVSFDLSFSAVKNFTEIASKFSERLRFDKSNPILLRMNDQLMFL
 ERAFIDPLGLPDRPFYRHVIYAPSSHNKYAGESFPGIYDALFDIESKVDP SQAWGEVKRQ
 ISIATFTVQAAAETLSEVA

SEQ ID NO: 56 Nucleotide sequence encoding the rhesus PSMA antigen of SEQ ID NO: 55

ATGGCTAGCGCTAGAAGGCCAGATGGCTGTGCGCTGGGCCCTGGTGTGGCTGGCGGATTCTT
 CCTGCTGGCTTCTCTGGCTGGTTCATCAAGTCCTCCAGCGAGGCCACCAACATCACCCCCA
 AGCACAACTGAAGGCCCTTCTGGACGAGCTGAAGGCCAGAAGATATCAAGAAGTTCTGCACAAAC
 TTCAACCCAGATCCCCAACCTGGCGGCCACCGAGCAGAACTTCAAGCTGGCCAAGCAGATCCAGTC
 CCAGTGGAAAGAGTTCGGCTGGACTCCGTGAACTGACCCACTACGACGTGCTGTCTTACCC
 CCAACAAAGACCCACCCAACTACATCTCCATCATCAACAGAGGCCAACGAAATCTTCAACACC
 TCCCTTGTGAGGCCACCCAGCGCTACAGAGAACCTGTGACCTGGCAGATCGTGCCTTCTCCGC
 ATTCAAGTCCACAAGGATGCCCCAGGGCGACCTGGTGTACGTGAACTACGCCAGGACCGAGGACT
 TCTTAAGCTGGAAAGGGACATGAAGATCAACTGCTCCGGCAAGATCGTATCGCCAGATA CGGC
 AAGGTGTTCAAGGGCAACAAAGTGAAGAACGCTCAGCTGGCTGGGCCACCGCGTGATCTGTA
 CTCTGACCCCGCAGACTTCTGCCAGGCGTGAAGTCTACCCGACGGCTGGAACCTGCG
 GTGGCGGAGTGCAGAGGGCAACATCTGAACACTGAACCGCCCTGGCGACCCCTGACCCAGGA
 TACCCGCCAACGAGTACGCCCTACAGAAGAGGAATGCCAGGGCTGGCCTGCCCCTATACCC
 AGTGAACCCATCGGCTACTACGACGCCAGAAACTGCTGGAAAAGATGGGGCTCCGCTCCC
 CCGACTCCCTGGAGAGGCTCTGAAGGTGCCATCACAGTGGGCCAGGGCTTACCGGCAC
 TTCTCCACCCAGAAAGTGAAGATGCACTCCACTCCACCTCGAAGTGAACGGATCTACAAACGT
 GATCGGCACCCCTGAGGAGGCCCTGGAAACCCGACAGATACTGATCTGGCGACCCAGGGACA
 GCTGGGTGTTGGCGGCATCGACCCACAGTCTGGCGCCTGTGGTGCACAGAGATCGTGCCTCC
 TTCGGAACCTGAAGAAGAGGGATGGCGCCAGAAGGAACATCTGTTGCCCTCTGGGACGC
 CGAGGAATTGGCCCTGCTGGGATCCACCGAGTGGGCCAGGAAAACCTCAGGTGCTGCAGGAAA
 GGGGCGTGCCTACATCAACCGCAGCTCTCCATCAGGGCAACTACACCTGAGGGTGGACTGC
 ACCCCCCCTGATGACTCTGGTGTACAACCTGACCAAGAGCTGGAATCCCCGACAGGGCTT
 CGAGGGCAAGTCTGTACGAGCTGGCAGCAAGAAGTCCCCTGGGAGTTCTCCGGCATATGC
 CCAGGATCTCAAAGCTGGGCTCGGCAACGACTTCGAGGTGTTCTCCAGAGGCTGGGAATGCC
 TCCGGCAGGGCCAGATAACCAAGAACTGGGAGACAAACAAGTTCTCTCTACCCCTGTACCA
 CTCCGTGTACGAAACCTACGAGCTGGTGGAAAAGTCTACGACCCCATGTTCAAGTACCACTG
 CGGTGGCCAGGGCATGGTGTGAGCTGGCAGCTGGCAACTCTGGTGTGGCTGCCCTTCGAC
 TGAGAGACTATGCTGTGGTGTGAGGAAGTACGCCGACAAATCTACACATCTCCATGAAGCA
 CCCCCAGGAAATGAAGACCTACTCCGTGTCTCGACTCCCTGTTCTCCGGCTGAAGAATTCA
 CCGAGATCGCCTCAAGTTCCGGAGGGCTGAGGGACTTCGACAAGTCCAACCCATCTGCTG
 AGGATGATGAAGCACCAGCTGATGTTCTGGAAAGGGCCTTCATCGACCCCTGGGCTGCCAGA
 CAGGGCCCTACAGGCCAGTACGCCCATCTCCCAACAAATACGCCGGAGTCC
 TCCCGGCATCTACGATGCCCTTGTGACATCGAGTCCAAGGTGGACCCCTCCAGGCTTGGGC
 GAAGTGAAGAGGGCAGATCAGTATGCCACATTCAAGTGCAAGGCCCTGCCGAAACCCCTGTC
 CGAAGTGGCC

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 62

<210> SEQ ID NO 1
 <211> LENGTH: 750
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

Met Trp Asn Leu Leu His Glu Thr Asp Ser Ala Val Ala Thr Ala Arg
 1 5 10 15

Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val Leu Ala Gly Gly Phe
 20 25 30

Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile Lys Ser Ser Asn Glu
 35 40 45

Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys Ala Phe Leu Asp Glu
 50 55 60

Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Gln Ile
 65 70 75 80

Pro His Leu Ala Gly Thr Glu Glu Asn Phe Gln Leu Ala Lys Gln Ile
 85 90 95

US 9,468,672 B2

175**176**

-continued

Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser Val Glu Leu Ala His
100 105 110

Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr Ile
115 120 125

Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu Phe
130 135 140

Glu Pro Pro Pro Gly Tyr Glu Asn Val Ser Asp Ile Val Pro Pro
145 150 155 160

Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val Tyr
165 170 175

Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp Met
180 185 190

Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys Val
195 200 205

Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala Lys Gly
210 215 220

Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val Lys
225 230 235 240

Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly Val Gln Arg Gly
245 250 255

Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly Tyr
260 265 270

Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val Gly
275 280 285

Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln Lys
290 295 300

Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp Arg
305 310 315 320

Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly Asn
325 330 335

Phe Ser Thr Gln Lys Val Lys Met His Ile His Ser Thr Asn Glu Val
340 345 350

Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu Pro
355 360 365

Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe Gly
370 375 380

Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val Arg
385 390 395 400

Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr Ile
405 410 415

Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser Thr
420 425 430

Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val Ala
435 440 445

Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val
450 455 460

Asp Cys Thr Pro Leu Met Tyr Ser Leu Val His Asn Leu Thr Lys Glu
465 470 475 480

Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu Ser
485 490 495

Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg Ile
500 505 510

Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg Leu

US 9,468,672 B2

177

178

-continued

515	520	525
Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr Asn		
530	535	540
Lys Phe Ser Gly Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr Glu		
545	550	555
Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys Tyr His Leu Thr Val		
565	570	575
Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile Val		
580	585	590
Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala		
595	600	605
Asp Lys Ile Tyr Ser Ile Ser Met Lys His Pro Gln Glu Met Lys Thr		
610	615	620
Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr		
625	630	635
640		
Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Phe Asp Lys Ser		
645	650	655
Asn Pro Ile Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu		
660	665	670
Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg		
675	680	685
His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser		
690	695	700
Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp		
705	710	715
720		
Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Tyr Val Ala Ala		
725	730	735
Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala		
740	745	750

<210> SEQ ID NO 2
<211> LENGTH: 2250
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

atgttggaaatc tccttcacgaaaccgactcg gctgtggcca cegcgcgcgcg cccgcgcgtgg 60
ctgtgcgcgtt gggcgcttgtt gctggcggtt ggcttctttc tccttcggcgtt ccttttcggg 120
tggtttataaa aatccctccaa tgaagctact aacattactc caaagcataaa tatgaaagca 180
tttttggatg aattgaaagc tgagaacatc aagaaggtttct tatataattt tacacagata 240
ccacatTTTtag cagggaaacaga acaaaacTTT cagcttgcAA agcaaattca atcccagtgg 300
aaagaatTTT gcctggatTC tgTTgagcta gcacattatg atgtcctgtt gtcctaccca 360
aaaataagactc atcccaacta catctcaata attaatgaa atggaaatga gatTTTcaac 420
acatcattat ttgaaaccacc tcctccagga tatgaaaatg tttcgatAT tgcgtaccacT 480
tttcagtgcTT tctctctca aggaatgcca gagggcgatc tagtgatgt taactatgca 540
cgaactgaaAG acttctttaa attggAACGG gacatgaaaa tcaattgtc tggggAAAATT 600
gtaaattgcca gatatgggaa agtttcaga gggaaataagg ttaaaaatgc ccagctggca 660
ggggccaaAG gagtcattCT ctactccgac cctgctgact actttgcTCC tgggggtGAAG 720
tccttatccag atgggtggaa tcttctggaa ggtgggtgtcc agcgtggaaa tatcctaaat 780
ctqaatqgtq caqqqaqaccc tctcacaccca qqttacccaq caaatqaata tqcttataqq 840

US 9,468,672 B2

179

180

-continued

cgtggaaattg cagaggctgt tggcttcca agtattcctg ttcatccat tggataactat 900
 gatgcacaga agtcctaga aaaaatgggt ggctcagcac caccagatag cagctggaga 960
 ggaagtctca aagtgccta caatgttga cctggctta ctggaaacctt ttctacacaa 1020
 aaagtcaaga tgcacatcca ctctaccaat gaagtgacaa gaatttacaa tgtgataggt 1080
 actctcagag gaggcgttga accagacaga tatgtcattc tgggaggtca ccgggactca 1140
 tgggtgtttg gtggatttga ccctcagagt ggaggcgttgc ttgttcatga aatttgagg 1200
 agctttggaa cactgaaaaa ggaagggtgg agacctagaa gaacaattt gtttgcaggc 1260
 tgggatgcag aagaatttgg tcttcttgg tctactgagt gggcagagga gaattcaaga 1320
 ctccctcaag agcgtggcgt ggcttatatt aatgctgact catctataga aggaaaactac 1380
 actctcagag ttgattgtac accgcgtatg tacagcttgg tacacaacct aacaaaagag 1440
 ctgaaaagcc ctgatgaagg cttaaaggc aaatctttt atgaaagtgg gactaaaaaa 1500
 agtccttccc cagagttcag tggcatgccc aggataagca aattggatc tggaaatgt 1560
 ttgaggtgt tcttccaaacg acttggattt gcttcaggca gagcacggta tactaaaaat 1620
 tgggaaacaa acaaatttcag cggctatcca ctgtatcaca gtgtctatga aacatatgag 1680
 ttgggtggaa agtttatga tccaatgttt aaatatcacc tcactgtggc ccagggtcg 1740
 ggagggatgg tgtttgcgtt agccaattcc atagtgtcc cttttgatgg tcgagattat 1800
 gctgtatgtt taagaaaagta tgctgacaaa atctacagta tttctatgaa acatccacag 1860
 gaaatgaaga catacagtgt atcatttgat tcacttttt ctgcagtaaa gaatttaca 1920
 gaaattgctt ccaagttcag tgagagactc caggacttgc aaaaaagcaa cccaatagta 1980
 tttaaatgtt tgaatgtca actcatgttt ctggaaagag catttattga tccatttaggg 2040
 ttaccagaca ggcctttta taggcatgtc atctatgctc caagcagccaa caacaagtat 2100
 gcaggggatgtt catccccagg aattttatgt gctctgtttt atattgaaag caaagtggac 2160
 ctttccaaagg cctggggaga agtgaagaga cagattttagt ttgcagccct cacagtgcag 2220
 gcagctgcag agacttttagt tgaatgtcc 2250

<210> SEQ ID NO 3

<211> LENGTH: 739

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: synthetic construct

<400> SEQUENCE: 3

Met	Ala	Ser	Ala	Arg	Arg	Pro	Arg	Trp	Leu	Cys	Ala	Gly	Ala	Leu	Val
1									5		10		15		

Leu	Ala	Gly	Gly	Phe	Phe	Leu	Leu	Gly	Phe	Leu	Phe	Gly	Trp	Phe	Ile
									20		25		30		

Lys	Ser	Ser	Ser	Glu	Ala	Thr	Asn	Ile	Ser	Pro	Gln	His	Asn	Val	Lys
									35		40		45		

Ala	Phe	Leu	Asp	Glu	Met	Lys	Ala	Glu	Asn	Ile	Lys	Lys	Phe	Leu	Tyr
					50				55		60				

Leu	Phe	Thr	Gln	Ile	Pro	His	Leu	Ala	Gly	Thr	Glu	Gln	Asn	Phe	Gln
					65				70		75		80		

Leu	Ala	Lys	Gln	Ile	Gln	Ala	Glu	Trp	Lys	Glu	Phe	Gly	Leu	Asp	Ser
									85		90		95		

Val	Glu	Leu	Ala	His	Tyr	Asp	Val	Leu	Leu	Ser	Tyr	Pro	Asn	Glu	Thr
									100		105		110		

-continued

His Pro Asn Tyr Ile Ser Ile Ile Asp Glu Asp Gly Asn Glu Ile Phe
115 120 125

Asn Thr Ser Leu Phe Glu Pro Pro Pro Pro Gly Tyr Glu Asn Ile Ser
130 135 140

Asp Val Val Pro Pro Tyr Ser Ala Phe Ser Pro Gln Gly Met Pro Glu
145 150 155 160

Gly Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys
165 170 175

Leu Glu Arg Glu Leu Lys Ile Asn Cys Ser Gly Lys Ile Leu Ile Ala
180 185 190

Arg Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu
195 200 205

Ala Gly Ala Lys Gly Ile Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe
210 215 220

Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly
225 230 235 240

Gly Val Gln Arg Gly Asn Val Leu Asn Leu Asn Gly Ala Gly Asp Pro
245 250 255

Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Glu Leu
260 265 270

Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr
275 280 285

Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro
290 295 300

Asp Ser Ser Trp Lys Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro
305 310 315 320

Gly Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met His Ile His
325 330 335

Ser Thr Asn Glu Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Ile Arg
340 345 350

Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp
355 360 365

Ala Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val
370 375 380

His Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Lys Gly Trp Arg
385 390 395 400

Pro Arg Arg Thr Ile Ile Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly
405 410 415

Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln
420 425 430

Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn
435 440 445

Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val Tyr
450 455 460

Asn Leu Thr Lys Glu Leu Gln Ser Pro Asp Glu Gly Phe Glu Gly Lys
465 470 475 480

Ser Leu Tyr Glu Ser Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe Ser
485 490 495

Gly Val Pro Arg Ile Asn Lys Leu Gly Ser Gly Asn Asp Phe Glu Val
500 505 510

Phe Phe Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys
515 520 525

-continued

Asn	Trp	Lys	Thr	Asn	Lys	Phe	Ser	Gly	Tyr	Pro	Leu	Tyr	His	Ser	Val
530						535					540				
Tyr	Glu	Thr	Tyr	Glu	Leu	Val	Glu	Lys	Phe	Tyr	Asp	Pro	Met	Phe	Lys
545					550			555							560
Tyr	His	Leu	Thr	Val	Ala	Gln	Val	Arg	Gly	Gly	Leu	Val	Phe	Glu	Leu
					565			570			575				
Ala	Asp	Ser	Ile	Val	Leu	Pro	Phe	Asp	Cys	Gln	Asp	Tyr	Ala	Val	Val
					580			585			590				
Leu	Arg	Lys	Tyr	Ala	Asp	Lys	Ile	Tyr	Asn	Leu	Ala	Met	Lys	His	Pro
					595			600			605				
Glu	Glu	Leu	Lys	Thr	Tyr	Ser	Val	Ser	Phe	Asp	Ser	Leu	Phe	Ser	Ala
					610			615			620				
Val	Lys	Asn	Phe	Thr	Glu	Ile	Ala	Ser	Lys	Phe	Asn	Gln	Arg	Leu	Gln
					625			630			635				640
Asp	Phe	Asp	Lys	Asn	Asn	Pro	Leu	Leu	Val	Arg	Met	Leu	Asn	Asp	Gln
					645			650			655				
Leu	Met	Phe	Leu	Glu	Arg	Ala	Phe	Val	Asp	Pro	Leu	Gly	Leu	Pro	Asp
					660			665			670				
Arg	Pro	Phe	Tyr	Arg	His	Val	Ile	Tyr	Ala	Pro	Ser	Ser	His	Asn	Lys
					675			680			685				
Tyr	Ala	Gly	Glu	Ser	Phe	Pro	Gly	Ile	Tyr	Asp	Ala	Leu	Phe	Asp	Ile
					690			695			700				
Glu	Ser	Lys	Val	Asp	Pro	Ser	Lys	Ala	Trp	Gly	Glu	Val	Lys	Arg	Gln
					705			710			715				720
Ile	Tyr	Val	Ala	Ala	Phe	Thr	Val	Gln	Ala	Ala	Ala	Glu	Thr	Leu	Ser
					725			730			735				

Glu Val Ala

```

<210> SEQ ID NO 4
<211> LENGTH: 2217
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 4
atggctagcg ccagacggcc cagatggctg tgcgccggag ccctgggtct ggccggagga 60
ttcttctgc tgggcttcct gttcggctgg ttcatcaaga gcagcagcga ggccaccaac 120
atcagcccccc agcacaacgt gaaggcctt ctggacgaga tgaaggccga gaacatcaag 180
aagtttctgt acctgttcac ccagatcccc cacctggccg gcaccgagca gaacttccag 240
ctggccaagc agattcaggc tgagtggaaa gagttcggcc tggacagcgt ggagctggcc 300
cactacgacg tgctgctgtc ctaccccaac gagacacacc ccaactacat cagcatcatc 360
gacgaggacg gcaacgagat tttcaacacc agcctgttc agccccctcc ccctggctac 420
gagaacatct ccgacgtggt gccccctac agcgccttca gccctcaggg aatgcctgaa 480
ggcgacacctg tgcgtacgtaa ctacgcccgg accgaggact tcttcaagct ggaacggag 540
ctgaagatca actgcagcgg caagatctgt atcgcacat acggcaaggt gttccggggc 600
aacaaagtga agaacgcaca gctggctgga gccaaggggca tcatcctgta cagcgacccc 660
ggcgactact tcgccccctgg cgtgaagtcc taccctgacg gctggAACCT gcctggccgc 720
ggagtgccgc gggcaacgt gctgaacctg aacggagccg gcgaccctct gaccccaggc 780
taccccgcca acgagtaacgc ctacggccgg gagctggccg aagccgtggg cctgcccagc 840

```

US 9,468,672 B2

185

186

-continued

atccccgtgc	accccatcg	ctactacgac	gcccagaaa	tgctggaaa	gatggggcgc	900
agegccccctc	ccgacagcag	cttgaaggc	agcctgaagg	tgcctacaa	cgtggccct	960
ggcttcaccg	gcaacttcag	caccagaaa	gtgaagatgc	acatccacag	caccaacgaa	1020
gtgacccgga	tctacaacgt	gatcgccacc	atcagaggcg	ccgtggagcc	cgacagatac	1080
gtgatcctgg	gcccgcaccg	ggacgcctgg	gtgttcggcg	gatcgaccc	ccagagcgga	1140
gcccgcgtgg	tgcaacgat	cgtgcggagc	tccggcaccc	tgaagaagaa	gggctggcg	1200
cccagacgga	ccatcattt	cgccagctgg	gacgcccagg	aattcggact	gctgggcct	1260
accgagtggg	ccgaggaaaa	cagcagactg	ctgcaggaa	ggggcgtcgc	ctacatcaac	1320
gccgacagct	ccatcgagg	caactacacc	ctgcgggtgg	actgcacccc	cctgatgtac	1380
agcctgtgt	acaacctgac	caaagagctg	cagagcccc	acgagggtt	cgagggcaag	1440
agcctgtacg	agagctggac	caagaagtcc	cccagcccc	agttcagcgg	cgtccccgg	1500
atcaacaagc	tggcagcgg	caacgacttc	gaggtgttct	tccagaggct	ggcattgcc	1560
agcggcagag	cccggtacac	caagaactgg	aaaaccaaca	agttctccgg	ctacccctg	1620
taccacacg	tgtacgagac	atcgaactg	gtggagaagt	tctacgaccc	catgttcaag	1680
taccacctga	ccgtggccca	ggtccgggga	gggctgggt	tgcactggc	cgacagcatc	1740
gtgctgcct	tcgactgcca	ggactatgct	gtgggtctgc	ggaagtacgc	cgacaaaatc	1800
tacaacctgg	ccatgaagca	ccccgaggaa	ctgaaaacct	acagcgtgtc	cttcgacagc	1860
ctgttcagcg	ccgtgaagaa	cttcacccgag	atgcacagca	agttcaacca	gcccgtcag	1920
gacttcgaca	agaacaaccc	cctgctggc	cggatgtga	acgaccagct	gatgttcctg	1980
gaacggccct	tcgtggaccc	cctggccctg	cctgaccggc	ccttctaccc	gcacgtgatc	2040
tatgccccca	gcagccacaa	caagtacgct	ggcgagagct	tcccccggat	ctacgatgcc	2100
ctgttcgaca	tcgagagcaa	ggtggacccc	agcaaggct	ggggcgaagt	gaagcggcag	2160
ataatacgtgg	ccgccttcac	agtgcaggcc	gctgccgaga	cactgagcga	ggtggcc	2217

<210> SEQ_ID NO 5

<211> LENGTH: 739

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 5

Met	Ala	Ser	Ala	Arg	Arg	Pro	Arg	Trp	Leu	Cys	Ala	Gly	Ala	Leu	Val
1									5					10	15

Leu	Ala	Gly	Gly	Phe	Phe	Leu	Leu	Gly	Phe	Leu	Phe	Gly	Trp	Phe	Ile
20									25					30	

Lys	Ser	Ser	Ser	Glu	Ala	Thr	Asn	Ile	Thr	Pro	Gln	His	Asn	Val	Lys
35									40					45	

Ala	Phe	Leu	Asp	Glu	Leu	Lys	Ala	Glu	Asn	Ile	Lys	Lys	Phe	Leu	Tyr
50									55					60	

Asn	Phe	Thr	Gln	Ile	Pro	His	Leu	Ala	Gly	Thr	Glu	Gln	Asn	Phe	Glu
65									70					75	80

Leu	Ala	Lys	Gln	Ile	Gln	Ala	Gln	Trp	Lys	Glu	Phe	Gly	Leu	Asp	Ser
85									90					95	

Val	Glu	Leu	Ser	His	Tyr	Asp	Val	Leu	Leu	Ser	Tyr	Pro	Asn	Glu	Thr
100									105					110	

His	Pro	Asn	Tyr	Ile	Ser	Ile	Ile	Asp	Glu	Asp	Gly	Asn	Glu	Ile	Phe
115									120					125	

-continued

Asn Thr Ser Leu Phe Glu Pro Pro Pro Pro Gly Tyr Glu Asn Ile Ser
 130 135 140
 Asp Val Val Pro Pro Tyr Ser Ala Phe Ser Pro Gln Gly Met Pro Glu
 145 150 155 160
 Gly Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys
 165 170 175
 Leu Glu Arg Asp Met Lys Ile Asn Cys Ser Gly Lys Ile Leu Ile Ala
 180 185 190
 Arg Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu
 195 200 205
 Ala Gly Ala Lys Gly Ile Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe
 210 215 220
 Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly
 225 230 235 240
 Gly Val Gln Arg Gly Asn Val Leu Asn Leu Asn Gly Ala Gly Asp Pro
 245 250 255
 Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile
 260 265 270
 Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr
 275 280 285
 Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ala Ala Pro Pro
 290 295 300
 Asp Ser Ser Trp Lys Gly Ser Leu Gln Val Pro Tyr Asn Val Gly Pro
 305 310 315 320
 Gly Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met His Ile His
 325 330 335
 Ser Thr Asn Glu Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Lys
 340 345 350
 Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp
 355 360 365
 Ala Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val
 370 375 380
 His Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Gly Trp Arg
 385 390 395 400
 Pro Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly
 405 410 415
 Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln
 420 425 430
 Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn
 435 440 445
 Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val Tyr
 450 455 460
 Asn Leu Thr Lys Glu Leu Gln Ser Pro Asp Glu Gly Phe Glu Gly Lys
 465 470 475 480
 Ser Leu Phe Asp Ser Trp Thr Glu Lys Ser Pro Ser Pro Glu Phe Ser
 485 490 495
 Gly Leu Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val
 500 505 510
 Phe Phe Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys
 515 520 525
 Asp Trp Lys Thr Ser Lys Phe Ser Gly Tyr Pro Leu Tyr His Ser Val
 530 535 540

-continued

Tyr Glu Thr Tyr Glu Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys
545 550 555 560

Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly Ile Val Phe Glu Leu
565 570 575

Ala Asn Ser Val Val Leu Pro Phe Asp Cys Gln Asp Tyr Ala Val Val
580 585 590

Leu Lys Lys Tyr Ala Asp Lys Ile Tyr Asn Ile Ser Met Lys His Pro
595 600 605

Gln Glu Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala
610 615 620

Val Lys Asn Phe Thr Glu Ile Ala Ser Lys Phe Asn Gln Arg Leu Gln
625 630 635 640

Asp Phe Asp Lys Asn Asn Pro Ile Leu Leu Arg Met Met Asn Asp Gln
645 650 655

Leu Met Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp
660 665 670

Arg Pro Phe Tyr Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys
675 680 685

Tyr Ala Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile
690 695 700

Glu Ser Lys Val Asp Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln
705 710 715 720

Ile Tyr Val Ala Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser
725 730 735

Glu Val Ala

<210> SEQ_ID NO 6
<211> LENGTH: 2217
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 6

```

atggctagcg ccagacggcc cagatggctg tggctggcg ccctgggtct ggctggccgc 60
ttttcctgc tggcttcct gttcggtctgg ttcatcaaga gcagcagcga ggccaccaac 120
atcacccccc agcacaacgt gaaggccctt ctggacgacg tgaaggccga gaatatcaag 180
aagttcctgt acaacttcac ccagatcccc cacctggccg gcaccgagca gaacttcgag 240
ctggccaaggc agatccaggc ccagtggaaa gagttcgcc tggacacgcgt ggaactgagc 300
caactacgacg tgctgctgag ctaccccaac gagacacacc ccaactacat cagcatcatc 360
gacgaggacg gcaacgagat tttcaacacc agcctgttcg agccccctcc accccggctac 420
gagaacatca ggcacgtggt gccccctac agcgcattca gtccacaggg aatgcggag 480
ggcgacctgg tgtacgtgaa ctacggccgg accgaggact ttttcaagct ggaacgggac 540
atgaagatca actgcagcgg caagatccgt atcgccagat acggcaaggt gttccggggc 600
aacaaagtga agaacgcccc gctggcaggc gccaagggc tcatcctgta cagcgacccc 660
gccgactact tcgccccctgg cgtgaagtcc taccccgacg gctggAACCT gcctggccgc 720
ggagtgacgaa ggggcaacgt gctgaacctg aacggcgctg ggcacccctt gacccctggc 780
taccccgccca acgagtagcgc ctacagacgg ggaatcgccg aggccgtggg cctgcgtac 840
atccctgtgc accccatcggt ctactacgac gcccagaaac tgctggaaaa gatggggcgg 900
ggccggccctc cccgacagctc ttggaaaggcgc agcctgcagg tccctacaa cgtggccct 960

```

-continued

```

ggtttcacccg gcaacttcag cacccagaaa gtgaagatgc acatccacag caccaacgaa 1020
gtgaccggga tctacaacgt gatcgccacc ctgaaggcgcc cgcttggaaacc cgacagatac 1080
gtgatccctgg gcccgcaccc ggacgcctgg gtgttggag gcatcgaccc tcagagcggc 1140
gtgcgcgtgg tgcacagat cgtgcggaccc ttccggcacac tgaagaagaa gggctggcgg 1200
ccccagacgga ccatacctgtt cgccagctgg gacgccgagg aattccggct gctggcagc 1260
accgagtggg ccgaggaaaa cagtcggctg ctgcaggaaac ggggcgtcgc ctacatcaac 1320
gccgacacgca gcatcgaggg caactacacc ctgcgggtgg actgcacccc cctgatgtac 1380
agcctgggtgt acaacactgac caaagagctg cagagccccc acgaggggctt cgaggggcaag 1440
tccctgttcg actcctggac cgagaagtcc cccagccccc agttcagcgg cctgeccaga 1500
atcagcaagc tgggcagcgg caacgacttc gaggtgttctt tccagggctt gggaaatcgcc 1560
agcggcagag cccggtacac caaggactgg aaaaccagca agttctccgg ctacccctg 1620
taccacacgc tgcacagac atacgagctg gtggaaaagt totacgaccc catgttcaag 1680
taccacactga ccgtggccca ggtccgaggg ggcacatcgatgt tccgactggc caacagcgt 1740
gtgctgcat tcgattgtca ggactacgac gtgggtgtga agaagtacgc cgacaaaatc 1800
tacaacatca gcatgaagca cccccagggaa atgaaaaaccc acagcgtgtc ctgcacacg 1860
ctgttcagcg ccgtgaagaa ttccaccggat atcgcctcca agttcaacca gagactgcag 1920
gacttcgaca agaacaaccc catttcgtgtc cggatgtga acgaccagct gatgttctg 1980
gaacgggcct tcategaccc cctgggcctg cccgacccggc ctttttaccc gcacgtgatc 2040
tatgccccca gcagccacaa caaatacggcc ggcgagagtt tcccccggcat ctacgtgcc 2100
ctgttcgata tcgagagcaa ggtggacccc agcaaggccctt ggggcgaatgt gaagcggcag 2160
atttacgtgg ccgcattcac agtgcaggct gctgccgaga cactgagcga ggtggcc 2217

```

<210> SEQ ID NO 7

<211> LENGTH: 739

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 7

Met	Ala	Ser	Ala	Arg	Arg	Pro	Arg	Trp	Leu	Cys	Ala	Gly	Ala	Leu	Val
1				5				10			15				

Leu	Ala	Gly	Gly	Phe	Phe	Leu	Leu	Gly	Phe	Leu	Phe	Gly	Trp	Phe	Ile
				20				25			30				

Lys	Ser	Ser	Asn	Glu	Ala	Thr	Asn	Ile	Thr	Pro	Lys	His	Asn	Met	Lys
				35				40			45				

Ala	Phe	Leu	Asp	Glu	Leu	Lys	Ala	Glu	Asn	Ile	Lys	Lys	Phe	Leu	Tyr
	50			55						60					

Asn	Phe	Thr	Gln	Ile	Pro	His	Leu	Ala	Gly	Thr	Glu	Gln	Asn	Phe	Gln
65				70				75			80				

Leu	Ala	Lys	Gln	Ile	Gln	Ser	Gln	Trp	Lys	Glu	Phe	Gly	Leu	Asp	Ser
				85				90			95				

Val	Glu	Leu	Ala	His	Tyr	Asp	Val	Leu	Leu	Ser	Tyr	Pro	Asn	Lys	Thr
							100			105			110		

His	Pro	Asn	Tyr	Ile	Ser	Ile	Ile	Asn	Glu	Asp	Gly	Asn	Glu	Ile	Phe
				115				120			125				

Asn	Thr	Ser	Leu	Phe	Glu	Pro	Pro	Pro	Gly	Tyr	Glu	Asn	Val	Ser
				130				135			140			

-continued

Asp Ile Val Pro Pro Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu
145 150 155 160

Gly Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys
165 170 175

Leu Glu Arg Asp Met Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala
180 185 190

Arg Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu
195 200 205

Ala Gly Ala Lys Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe
210 215 220

Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly
225 230 235 240

Gly Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro
245 250 255

Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile
260 265 270

Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr
275 280 285

Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro
290 295 300

Asp Ser Ser Trp Arg Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro
305 310 315 320

Gly Phe Thr Gly Asn Phe Ser Ala Gln Lys Leu Lys Leu His Ile His
325 330 335

Ser Asn Thr Lys Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg
340 345 350

Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp
355 360 365

Ser Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val
370 375 380

His Glu Ile Val Arg Thr Phe Gly Thr Leu Lys Lys Gly Trp Arg
385 390 395 400

Pro Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly
405 410 415

Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln
420 425 430

Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn
435 440 445

Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Leu His Ser Leu Val Tyr
450 455 460

Asn Leu Thr Lys Glu Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys
465 470 475 480

Ser Leu Tyr Glu Ser Trp Thr Lys Lys Ser Pro Ser Pro Glu Leu Ser
485 490 495

Gly Leu Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val
500 505 510

Phe Phe Gln Arg Leu Gly Ile Ser Ser Gly Arg Ala Arg Tyr Thr Lys
515 520 525

Asp Trp Lys Thr Ser Lys Phe Ser Ser Tyr Pro Leu Tyr His Ser Ile
530 535 540

Tyr Glu Thr Tyr Glu Leu Val Val Lys Phe Tyr Asp Pro Met Phe Lys
545 550 555 560

-continued

Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu
565 570 575

Ala Asn Ser Ile Val Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Ala
580 585 590

Leu Lys Asn His Ala Glu Asn Leu Tyr Ser Ile Ser Met Lys His Pro
595 600 605

Gln Glu Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala
610 615 620

Val Lys Asn Phe Thr Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln
625 630 635 640

Asp Phe Asp Lys Ser Asn Pro Ile Val Leu Arg Met Met Asn Asp Gln
645 650 655

Leu Met Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp
660 665 670

Arg Pro Phe Tyr Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys
675 680 685

Tyr Ala Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile
690 695 700

Glu Ser Lys Val Asp Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln
705 710 715 720

Ile Tyr Val Ala Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser
725 730 735

Glu Val Ala

<210> SEQ ID NO 8

<211> LENGTH: 2217

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 8

```

atggctagcg ccagacggcc cagatggctg tggctggcg ccctgggtgc ggctggccgc 60
tttttctgc tgggttcct gttcggctgg ttcatcaaga gcagcaacga ggccaccaac 120
atcaccccca agcacaacat gaaggccctt ctggacgagc tgaaggccga gaatatcaag 180
aagtcttgt acaacttcac ccagatcccc cacctggccg gcaccgagca gaacttcag 240
ctggccaagc agatccagag ccagtggaaa gagttcgcc tggacagcgt ggaactggcc 300
caactacgac tgctgctgag ctacccaaac aagacccacc ccaactacat cagcatcatc 360
aacgaggacg gcaacgagat tttcaacacc agcctgttcg agccccctcc accccggctac 420
gagaacgtgt ccgacatcgt gccccattc agcgcattca gtccacaggg aatgcccag 480
ggcgacatgg tggatgtgaa ctacgcccgg accgaggact tcttcaagct ggaacgggac 540
atgaagatca actgcagcgg caagatcgtg atgcgcagat acggcaaggt gttccgggc 600
aacaaggatga agaacgcccc gctggcaggc gccaaggccg tgatcctgta tagcgcaccc 660
gccgactact tcgccccctgg cgtgaagtcc taccccgacg gctggAACCT gcctggccgc 720
ggagtgcagc gggcaacat cctgaacctg aacggcgctg gogaccocct gaccctggc 780
tatcccccca acgagtagcgc ctacagacgg ggaatcgccc aggccgtggg cctgccttagc 840
atccctgtgc accccatcgg ctactacgac gcccagaaac tgctggaaaa gatggggccgc 900
agcgcacccctc ccgatagctc ttggagagggc agcctgaagg tgccctacaa cgtggccct 960
ggcttcacccg gcaacttcag cgcccaagaag ctgaagctgc acatccacag caacacccaa 1020

```

-continued

gtgaccggta tctacaacgt gatcgccacc ctgagaggcg ccgttggaaacc cgacagatac 1080
 gtgatcctgg gcccgcaccc ggacagctgg gtgttcggcc gcatcgaccc tcagtctggc 1140
 gccgctgtgg tgcacgat cgtcgccacc tttggcaccc tgaagaagaa gggctggcg 1200
 cccagacgga ccatacgtt cgccagctgg gacgccgagg aattccggct gctggcagc 1260
 accgagtgcc cgaggaaaaa cagtcggctg ctgcaggAAC gggcgctcgct acatcaac 1320
 gccgacacga gcatecgaggg caactacacc ctgcgggtgg actgcacccc cctgtgcac 1380
 agcctggtgt acaacctgac caaagagctg aagtcccccc acgaggggctt cgagggcaag 1440
 agcctgtacg agagctggac caagaagtcc cccagcccc agctgagccg cctgcccaga 1500
 atcagcaacg tggcagccg caacgacttc gaggtgttct tccagcgct gggcatcagc 1560
 agcggcagag cccggtagac caaggactgg aaaaccagca agttcagcag ctacccctg 1620
 taccacagca tctacgagac atacgagctg gtggtaagt tctacgaccc catgttcaag 1680
 taccacactga ccgtggccca ggtccgaggc ggcattgggt tcgagctggc caacagcatc 1740
 gtgctgcct tcgactgcgc ggactacgcc gtggccctga agaaccacgc cgagaacctg 1800
 tacagcatca gcatgaagca cccccaggaa atgaaaacct acagcgtgtc cttcgacacg 1860
 ctgttcagcg ccgtgaagaa tttcaccgag atcgcctcca agttcagcga gcccgtgcag 1920
 gacttcgaca agagcaaccc catcgtgtc agaatgtatc acgaccagct gatgttctg 1980
 gaacgggcct tcatcgaccc cctggccctg cccgaccggc ctttttaccg gcacgtgtc 2040
 tatgccccca gcagccacaa caaatacgcg ggcgagagt tccccggcat ctacgatgcc 2100
 ctgttcgaca tcgagagcaa ggtggacccc agcaaggccct ggggcgaagt gaagcggcag 2160
 atttacgtgg ccgcattcac agtgcaggcc gctgccgaga cactgagcga ggtggcc 2217

<210> SEQ ID NO 9

<211> LENGTH: 739

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 9

Met Ala Ser Ala Arg Arg Pro Arg Trp Leu Cys Ala Gly Ala	Lue Val		
1	5	10	15

Lue Ala Gly Phe Phe Leu Lue Gly Phe Lue Phe Gly Trp Phe Ile		
20	25	30

Lys Ser Ser Asn Glu Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys		
35	40	45

Ala Phe Leu Asp Glu Lue Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr		
50	55	60

Asn Phe Thr Gln Ile Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln			
65	70	75	80

Leu Ala Lys Gln Ile Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser		
85	90	95

Val Glu Leu Ala His Tyr Asp Val Leu Lue Ser Tyr Pro Asn Lys Thr		
100	105	110

His Pro Asn Tyr Ile Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe		
115	120	125

Asn Thr Ser Leu Phe Glu Pro Pro Pro Gly Tyr Glu Asn Val Ser		
130	135	140

Asp Ile Val Pro Pro Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu			
145	150	155	160

US 9,468,672 B2

199

-continued

Gly Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys
 165 170 175

Leu Glu Arg Asp Met Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala
 180 185 190

Arg Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu
 195 200 205

Ala Gly Ala Lys Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe
 210 215 220

Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly
 225 230 235 240

Gly Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro
 245 250 255

Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile
 260 265 270

Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr
 275 280 285

Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Pro Pro
 290 295 300

Asp Ser Ser Trp Arg Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro
 305 310 315 320

Gly Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met His Ile His
 325 330 335

Ser Thr Asn Glu Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg
 340 345 350

Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp
 355 360 365

Ser Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val
 370 375 380

His Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg
 385 390 395 400

Pro Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly
 405 410 415

Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln
 420 425 430

Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn
 435 440 445

Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val His
 450 455 460

Asn Leu Thr Lys Glu Leu Lys Ser Pro Asp Glu Gly Phe Glu Gly Lys
 465 470 475 480

Ser Leu Tyr Glu Ser Trp Thr Lys Lys Ser Pro Ser Pro Glu Phe Ser
 485 490 495

Gly Met Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val
 500 505 510

Phe Phe Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys
 515 520 525

Asn Trp Glu Thr Asn Lys Phe Ser Gly Tyr Pro Leu Tyr His Ser Val
 530 535 540

Tyr Glu Thr Tyr Glu Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys
 545 550 555 560

Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu
 565 570 575

200

-continued

Ala Asn Ser Ile Val Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val
 580 585 590

Leu Arg Lys Tyr Ala Asp Lys Ile Tyr Ser Ile Ser Met Lys His Pro
 595 600 605

Gln Glu Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala
 610 615 620

Val Lys Asn Phe Thr Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Gln
 625 630 635 640

Asp Phe Asp Lys Ser Asn Pro Ile Val Leu Arg Met Met Asn Asp Gln
 645 650 655

Leu Met Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp
 660 665 670

Arg Pro Phe Tyr Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys
 675 680 685

Tyr Ala Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile
 690 695 700

Glu Ser Lys Val Asp Pro Ser Lys Ala Trp Gly Glu Val Lys Arg Gln
 705 710 715 720

Ile Tyr Val Ala Ala Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser
 725 730 735

Glu Val Ala

<210> SEQ ID NO 10

<211> LENGTH: 2217

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 10

atggctagcg	cgcgcgcccc	gchgctggctg	tgcgctgggg	cgctgggtct	ggcggtggc	60
ttcttttcc	tcggcttcct	cttcgggtgg	tttataaaat	cctccaatga	agctactaac	120
attactccaa	agcataatat	gaaagcattt	ttggatgaat	tgaaagctga	gaacatcaag	180
aagttcttat	ataatttac	acagatacca	catttagcag	gaacagaaca	aaactttcag	240
cttgcaaaggc	aaattcaatc	ccagtggaaa	gaatttggcc	tggattctgt	tgagctggca	300
cattatgatg	tcctgttgtc	ctacccaaat	aagactcatac	ccaactacat	ctcaataatt	360
aatgaagatg	gaaatgagat	tttcaacaca	tcattatgg	aaccacctcc	tccaggatat	420
gaaaaatgtt	cgatattgt	accacccccc	agtgcattct	ctccctcaagg	aatgccagag	480
ggcgatctag	tgtatgttaa	ctatgcacga	actgaagact	tctttaaattt	ggaacgggac	540
atgaaaatca	attgctctgg	gaaaattgtta	attgccagat	atgggaaagt	tttcagagga	600
aataagggtta	aaaatgcccc	gctggcagggg	gccaaaggag	tcattctcta	ctccgaccct	660
gtgactact	ttgctctgg	ggtaagtcc	tatccagatg	gttggaatct	tcctggaggt	720
ggtgtccagc	gtggaaatat	cctaaatctg	aatggtgca	gagaccctct	cacaccagg	780
tacccagcaa	atgaatatgc	tttagggct	ggaatttgca	aggctgttgg	tcttccaagt	840
atccctgttc	atccaaatgg	atactatgt	gcacagaagc	tccttagaaaa	aatgggtggc	900
tcagcaccac	cagatagcag	ctggagagga	agtctcaaag	tgcctacaa	tgttggaccc	960
ggctttactg	gaaacttttc	tacacaaaaa	gtcaagatgc	acatccactc	taccaatgaa	1020
gtgacaagaa	tttacaatgt	gataggtact	ctcagaggag	cagtggaaacc	agacagatat	1080
gtcattctgg	gaggtcacccg	ggactcatgg	gtgtttgggtg	gtattgaccc	tcagagtgg	1140

-continued

```

gcagctgttg ttcatgaaat tgtgaggagc tttggAACAC tgaaaaAGGA agggTggaga 1200
ccatTTGTT tgcaAGCTGG gatgcAGAAg aATTTGGTCT tCTTGTTCT 1260
actgAGTGGG cAGAGGAGAA ttCAAGACTC ctTCAGAGC gtggcGTggC ttatattaAT 1320
gtGTACTCAT ctatAGAAGG aaACTACACT ctGAGAGTTG attGTACACC gCTGTGTAC 1380
agCTTGGTAC aCAACCTAAC AAAAGAGCTG AAAAGCCCTG atGAAGGCTT tGAAGGCAA 1440
tCTCTTATG aaAGTTGGAC tAAAAAAAAGT cTTCCCCAG agTTCAgTGG catGCCAGG 1500
ataAGCAAT tGGGATCTGG aaATGATTT gagGTGTTCT tCCAACGACT tGGAATTGCT 1560
tcAGGCAGAG cACGGTATAc tAAAATTGG gAAACAAACA aATTCAgCGG CTATCCACTG 1620
tatCACAGTG tCTATGAAAC atATGAGTTG gtGAAAAGT tTTATGATCC aATGTTAAA 1680
tatCACCTCA ctGTGGCCCA ggTTGAGGA gGGATGGTGT ttGAGTGGC caATTCATA 1740
gtGCTCCCTT ttGATTGTCG agATTATGCT gtAGTTAA gAAAGTATGC tgACAAATC 1800
taCAGTATTt CTATGAAACA tCCACAGGAA atGAAGACAT acAGTGTATC attTGATTCA 1860
ctTTTTCTG cAGTAAGAA tTTTACAGAA attGCTTCCA agTTCAgTGA gagACTCCAG 1920
gaCTTTGACA AAAGCAACCC aATAGTATTA agAAATGATGA atGATCACT catTTCTG 1980
gAAAGAGCAT ttATTGATCC attAGGGTTA ccAGACAGGC ctTTTTATAG gCATGTcATC 2040
tatGCTCCAA gcAGCCACAA caAGTATGCa gGGGAGTCA tCCAGGAAT ttATGATGCT 2100
ctGTTTgATA ttGAAAGCAA agTGGACCCt tCCAAGGCt gGGGAGAAGt gaAGAGACAG 2160
atttATGTTG cAGCCTTCAC agTGCAGGCA gCTGCAgAGA CTTGAGTGA agTAGCC 2217

```

<210> SEQ ID NO 11

<211> LENGTH: 710

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 11

Met	Ala	Ser	Lys	Ser	Ser	Asn	Glu	Ala	Thr	Asn	Ile	Thr	Pro	Lys	His
1								5		10			15		

Asn	Met	Lys	Ala	Phe	Leu	Asp	Glu	Leu	Lys	Ala	Glu	Asn	Ile	Lys	Lys
				20				25				30			

Phe	Leu	Tyr	Asn	Phe	Thr	Gln	Ile	Pro	His	Leu	Ala	Gly	Thr	Glu	Gln
				35				40				45			

Asn	Phe	Gln	Leu	Ala	Lys	Gln	Ile	Gln	Ser	Gln	Trp	Lys	Glu	Phe	Gly
				50				55			60				

Leu	Asp	Ser	Val	Glu	Leu	Ala	His	Tyr	Asp	Val	Leu	Leu	Ser	Tyr	Pro
				65				70			75			80	

Asn	Lys	Thr	His	Pro	Asn	Tyr	Ile	Ser	Ile	Ile	Asn	Glu	Asp	Gly	Asn
				85				90			95				

Glu	Ile	Phe	Asn	Thr	Ser	Leu	Phe	Glu	Pro	Pro	Pro	Gly	Tyr	Glu
				100				105				110		

Asn	Val	Ser	Asp	Ile	Val	Pro	Pro	Phe	Ser	Ala	Phe	Ser	Pro	Gln	Gly
				115				120			125				

Met	Pro	Glu	Gly	Asp	Leu	Val	Tyr	Val	Asn	Tyr	Ala	Arg	Thr	Glu	Asp
				130				135			140				

Phe	Phe	Lys	Leu	Glu	Arg	Asp	Met	Lys	Ile	Asn	Cys	Ser	Gly	Lys	Ile
145								150			155			160	

Val	Ile	Ala	Arg	Tyr	Gly	Lys	Val	Phe	Arg	Gly	Asn	Lys	Val	Lys	Asn
				165				170			175				

US 9,468,672 B2

205**206**

-continued

Ala Gln Leu Ala Gly Ala Lys Gly Val Ile Leu Tyr Ser Asp Pro Ala
 180 185 190

Asp Tyr Phe Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu
 195 200 205

Pro Gly Gly Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala
 210 215 220

Gly Asp Pro Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg
 225 230 235 240

Arg Gly Ile Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro
 245 250 255

Ile Gly Tyr Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser
 260 265 270

Ala Pro Pro Asp Ser Ser Trp Arg Gly Ser Leu Lys Val Pro Tyr Asn
 275 280 285

Val Gly Pro Gly Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met
 290 295 300

His Ile His Ser Thr Asn Glu Val Thr Arg Ile Tyr Asn Val Ile Gly
 305 310 315 320

Thr Leu Arg Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly
 325 330 335

His Arg Asp Ser Trp Val Phe Gly Ile Asp Pro Gln Ser Gly Ala
 340 345 350

Ala Val Val His Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Glu
 355 360 365

Gly Trp Arg Pro Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu
 370 375 380

Glu Phe Gly Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg
 385 390 395 400

Leu Leu Gln Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile
 405 410 415

Glu Gly Asn Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser
 420 425 430

Leu Val His Asn Leu Thr Lys Glu Leu Lys Ser Pro Asp Glu Gly Phe
 435 440 445

Glu Gly Lys Ser Leu Tyr Glu Ser Trp Thr Lys Lys Ser Pro Ser Pro
 450 455 460

Glu Phe Ser Gly Met Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp
 465 470 475 480

Phe Glu Val Phe Phe Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg
 485 490 495

Tyr Thr Lys Asn Trp Glu Thr Asn Lys Phe Ser Gly Tyr Pro Leu Tyr
 500 505 510

His Ser Val Tyr Glu Thr Tyr Glu Leu Val Glu Lys Phe Tyr Asp Pro
 515 520 525

Met Phe Lys Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly Met Val
 530 535 540

Phe Glu Leu Ala Asn Ser Ile Val Leu Pro Phe Asp Cys Arg Asp Tyr
 545 550 555 560

Ala Val Val Leu Arg Lys Tyr Ala Asp Lys Ile Tyr Ser Ile Ser Met
 565 570 575

Lys His Pro Gln Glu Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu
 580 585 590

US 9,468,672 B2

207

208

-continued

Phe	Ser	Ala	Val	Lys	Asn	Phe	Thr	Glu	Ile	Ala	Ser	Lys	Phe	Ser	Glu
595						600						605			

Arg	Leu	Gln	Asp	Phe	Asp	Lys	Ser	Asn	Pro	Ile	Val	Leu	Arg	Met	Met
610						615						620			

Asn	Asp	Gln	Leu	Met	Phe	Leu	Glu	Arg	Ala	Phe	Ile	Asp	Pro	Leu	Gly
625					630			635				640			

Leu	Pro	Asp	Arg	Pro	Phe	Tyr	Arg	His	Val	Ile	Tyr	Ala	Pro	Ser	Ser
645					650			655				655			

His	Asn	Lys	Tyr	Ala	Gly	Glu	Ser	Phe	Pro	Gly	Ile	Tyr	Asp	Ala	Leu
660					665						670				

Phe	Asp	Ile	Glu	Ser	Lys	Val	Asp	Pro	Ser	Lys	Ala	Trp	Gly	Glu	Val
675					680			685				685			

Lys	Arg	Gln	Ile	Tyr	Val	Ala	Ala	Phe	Thr	Val	Gln	Ala	Ala	Ala	Glu
690					695			700				700			

Thr	Leu	Ser	Glu	Val	Ala
705				710	

<210> SEQ_ID NO 12

<211> LENGTH: 2130

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 12

atggctagca	aatcctccaa	tgaagctact	aacattactc	caaaggataa	tatgaaagca	60
tttttggatg	aattgaaagc	tgagaacatc	aagaagtct	tatataattt	tacacagata	120
ccacatttag	caggaacaga	acaaaacttt	cagcttgcaa	agcaaattca	atcccagtgg	180
aaagaatttg	gcctggattc	tgttgagtc	gcacattatg	atgtccctgtt	gtcctaccca	240
aataagactc	atcccaacta	catctcaata	attaatgaag	atggaaatga	gattttcaac	300
acatcattat	ttgaaccacc	tcctccagga	tatgaaaatg	tttcggatat	tgtaccacct	360
ttcagtgcctt	tctctcctca	aggaatgccca	gaggcgatc	tagtgtatgt	taactatgca	420
cgaactgaag	acttctttaa	attggaacgg	gacatgaaaa	tcaattgctc	tgggaaaatt	480
gtaattgcca	gatatggaa	agtttcaga	gaaaataagg	ttaaaaatgc	ccagctggca	540
ggggccaaag	gagtcattct	ctactccgac	cctgctgact	actttgctcc	tggggtgaag	600
tcctatccag	atgggtggaa	tcttcctgga	ggtgggttcc	agcgtggaaa	tatcctaaat	660
ctgaatggtg	caggagaccc	tctcacacca	ggttacccag	caaataata	tgcttatagg	720
cgtggaattg	cagaggctgt	tggtcttcca	agtattcctg	ttcattcaat	tggatactat	780
gatgcacaga	agtccttaga	aaaaatgggt	ggtcagcac	caccagatag	cagctggaga	840
ggaagtctca	aagtgcctca	caatgttgg	cctggcttta	ctggaaactt	ttctacacaa	900
aaagtcaaga	tgcacatcca	ctctaccaat	gaagtgcacaa	gaatttacaa	tgtgataggt	960
actctcagag	gagcagtgaa	accagacaga	tatgtcattc	tgggaggatca	ccgggactca	1020
tgggtgtttg	tggttattga	ccctcagagt	ggagcagctg	ttgttcatga	aattgtgagg	1080
agctttggaa	cactgaaaaa	ggaagggtgg	agacctagaa	gaacaatttt	gtttgcaagc	1140
tgggatgcag	aagaatttgg	tcttcctggt	tctactgagt	gggcagagga	gaattcaaga	1200
ctccttcaga	agcgtggcgt	ggcttatatt	aatgctgact	catctataga	aggaaactac	1260
actctcagag	ttgattgtac	accgctgatg	tacagcttg	tacacaacct	aacaaaagag	1320
ctgaaaagcc	ctgatgaagg	catttgcaggc	aaatctcttt	atgaaaagtg	gactaaaaaa	1380

-continued

```

agtcctcccc cagagttcag tggcatgccccc aggataagca aattgggatc tggaaatgtat 1440
tttgagggtgt ttttccaacg acttggaaatt gtttcaggca gagcacggta tactaaaaat 1500
tgggaaacaa acaaattcag cggctatcca ctgttatcaca gtgtctatga aacatatgag 1560
ttgggtggaaa agtttatga tccaatgttt aaatatcacc tcactgtggc ccaggttcga 1620
ggagggatgg tggttgagct ggccaattcc atagtgcctt ctttgatttgcagattat 1680
gctgttagttt taagaaagta tgctgacaaa atctacagta ttcttatgaa acatccacag 1740
gaaatgaaga catacagtgt atcatttgat tcactttttt ctgcagtaaa gaattttaca 1800
gaaattgctt ccaagttcag tgagagactc caggacttgc acaaaggca cccaaatagta 1860
ttaagaatga tgaatgatca actcatgttt ctggaaagag cattttatgaa tccatttaggg 1920
ttaccagaca ggcctttta taggcattgtc atctatgcctt caagcagccca caacaagttat 1980
gcagggggagt cattcccgagg aatttatgtat gctctgttttgcatttgc 2040
ccttccaagg cctgggggaga agtgaagaga cagattttatgc ttgcagccctt cacagtgcag 2100
gcagctgcag agacttttagt gtaagtagcc 2130

```

<210> SEQ ID NO 13

<211> LENGTH: 732

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 13

Met	Ala	Ser	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Trp
1							5				10			15

Val	Pro	Gly	Ser	Thr	Gly	Asp	Ala	Ala	Lys	Ser	Ser	Asn	Glu	Ala	Thr
							20			25			30		

Asn	Ile	Thr	Pro	Lys	His	Asn	Met	Lys	Ala	Phe	Leu	Asp	Glu	Leu	Lys
							35			40			45		

Ala	Glu	Asn	Ile	Lys	Lys	Phe	Leu	Tyr	Asn	Phe	Thr	Gln	Ile	Pro	His
							50			55			60		

Leu	Ala	Gly	Thr	Glu	Gln	Asn	Phe	Gln	Leu	Ala	Lys	Gln	Ile	Gln	Ser
							65			70			75		80

Gln	Trp	Lys	Glu	Phe	Gly	Leu	Asp	Ser	Val	Glu	Leu	Ala	His	Tyr	Asp
							85			90			95		

Val	Leu	Leu	Ser	Tyr	Pro	Asn	Lys	Thr	His	Pro	Asn	Tyr	Ile	Ser	Ile
							100			105			110		

Ile	Asn	Glu	Asp	Gly	Asn	Glu	Ile	Phe	Asn	Thr	Ser	Leu	Phe	Glu	Pro
							115			120			125		

Pro	Pro	Pro	Gly	Tyr	Glu	Asn	Val	Ser	Asp	Ile	Val	Pro	Pro	Phe	Ser
							130			135			140		

Ala	Phe	Ser	Pro	Gln	Gly	Met	Pro	Glu	Gly	Asp	Leu	Val	Tyr	Val	Asn
							145			150			155		160

Tyr	Ala	Arg	Thr	Glu	Asp	Phe	Phe	Lys	Leu	Glu	Arg	Asp	Met	Lys	Ile
							165			170			175		

Asn	Cys	Ser	Gly	Lys	Ile	Val	Ile	Ala	Arg	Tyr	Gly	Lys	Val	Phe	Arg
							180			185			190		

Gly	Asn	Lys	Val	Lys	Asn	Ala	Gln	Leu	Ala	Gly	Ala	Lys	Gly	Val	Ile
							195			200			205		

Leu	Tyr	Ser	Asp	Pro	Ala	Asp	Tyr	Phe	Ala	Pro	Gly	Val	Lys	Ser	Tyr
							210			215			220		

-continued

Pro Asp Gly Trp Asn Leu Pro Gly Gly Val Gln Arg Gly Asn Ile
 225 230 235 240
 Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly Tyr Pro Ala
 245 250 255
 Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val Gly Leu Pro
 260 265 270
 Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln Lys Leu Leu
 275 280 285
 Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp Arg Gly Ser
 290 295 300
 Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly Asn Phe Ser
 305 310 315 320
 Thr Gln Lys Val Lys Met His Ile His Ser Thr Asn Glu Val Thr Arg
 325 330 335
 Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu Pro Asp Arg
 340 345 350
 Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe Gly Ile
 355 360 365
 Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val Arg Ser Phe
 370 375 380
 Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr Ile Leu Phe
 385 390 395 400
 Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser Thr Glu Trp
 405 410 415
 Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val Ala Tyr Ile
 420 425 430
 Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val Asp Cys
 435 440 445
 Thr Pro Leu Met Tyr Ser Leu Val His Asn Leu Thr Lys Glu Leu Lys
 450 455 460
 Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu Ser Trp Thr
 465 470 475 480
 Lys Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg Ile Ser Lys
 485 490 495
 Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg Leu Gly Ile
 500 505 510
 Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr Asn Lys Phe
 515 520 525
 Ser Gly Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr Glu Leu Val
 530 535 540
 Glu Lys Phe Tyr Asp Pro Met Phe Lys Tyr His Leu Thr Val Ala Gln
 545 550 555 560
 Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile Val Leu Pro
 565 570 575
 Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala Asp Lys
 580 585 590
 Ile Tyr Ser Ile Ser Met Lys His Pro Gln Glu Met Lys Thr Tyr Ser
 595 600 605
 Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr Glu Ile
 610 615 620
 Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Phe Asp Lys Ser Asn Pro
 625 630 635 640
 Ile Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu Arg Ala

-continued

645	650	655
Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg His Val		
660	665	670
Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser Phe Pro		
675	680	685
Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp Pro Ser		
690	695	700
Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Tyr Val Ala Ala Phe Thr		
705	710	715
Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala		
725	730	

<210> SEQ ID NO 14

<211> LENGTH: 2196

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 14

atggctagcg aaacggacac tttgttgg tgggtgttt tgctttgggt acccgatct	60
actggtgatg ctgctaaatc ctc当地 gactactaaca ttactccaaa gcataatatg	120
aaagcatttt tggatgaatt gaaagcttag aacatcaaga agttcttata taatttaca	180
cagataaccac atttagcagg aacagaacaa aacttcagc ttgcaaagca aattcaatcc	240
cagtggaaag aatttgcct ggattctgtt gagcttagcac attatgtatgt cctgttgtcc	300
tacccaaata agactcatcc caactacatc tcaataatta atgaagatgg aaatgagatt	360
ttcaacacat cattatttga accacctctt ccaggatatg aaaatgtttc ggatattgtta	420
ccaccttca gtgctttctc tc当地 agggcagg ggatcttagt gtatgttaac	480
tatgcacgaa ctgaagactt ct当地 aacgggaca tgaaaatcaa ttgctctggg	540
aaaattgtaa ttgccagata tggaaagtt ttcagaggaa ataaggtaa aaatgccag	600
ctggcagggg ccaaaggagt cattctctac tccgaccctg ctgactactt tgctctggg	660
gtgaagtccct atccagatgg tt当地 cctggagggtg gtgtccagcg tggaaatatc	720
ctaaatctga atggcagg agaccctctc acaccaggat acccagcaaa tgaatatgct	780
tataggcgtg gaattgcaga ggctgttgg tttccaagta tt当地 tccaaatttggaa	840
tactatgtatc cacagaagct ccttagaaaa atgggtggct cagcaccacc agatagcagc	900
tggagaggaa gtctcaaagt gccctacaat gttggacctg gctttactgg aaactttct	960
acacaaaaag tcaagatgca catccactct accaatgaag tgacaagaat ttacaatgtg	1020
ataggactc tcaaggaggc agtggacca gacagatatg tcattctggg aggtcacccg	1080
gactcatggg tggcttggg tattgaccct cagatggag cagctgttgt tcatgaaatt	1140
gtgaggagct tt当地 acact gaaaaaggaa gggtggagac ct当地 aacatggatgtt	1200
gcaagctggg atgcagaaga atttggctt cttggctca ctgagtgccc agaggagaat	1260
tcaagactcc tt当地 agaggcgtg tggcttggct tatattaatg ctgactcatc tatagaaggg	1320
aactacactc tgagagttga tt当地 acaccg ctgatgtaca gcttggtaca caacctaaca	1380
aaagagctga aaagccctga tgaaggctt gaaggcaat ctctttatga aagttggact	1440
aaaaaaaaatgc cttcccccaga gttcagtgcc atgcccaggaa taagcaattt gggatctggaa	1500
aatgattttgg aggtgttctt ccaacgactt ggaattgtt caggcagagc acggataact	1560

-continued

```

aaaaattggg aaacaaacaa attcagccgc tatccactgt atcacagtgt ctatgaaaca 1620
tatgagttgg tggaaaagtt ttatgatcca atgtttaaat atcacctcac tgtggccag 1680
gttcgaggag gcatgggttt tgagctagec aattccatag tgctccctt tgattgtcga 1740
gattatgctg tagtttaag aaagtatgtc gacaaaatct acagtatttc tatgaaacat 1800
ccacaggaaa tgaagacata cagtgatca tttgattcac tttttctgc agtaaagaat 1860
tttacagaaa ttgcttccaa gttcgtgag agactccagg actttgacaa aagcaaccca 1920
atagtattaa gaatgtatgaa tcatcaactc atgtttctgg aaagagcatt tattgatcca 1980
tttagggttac cagacaggcc ttttatagg catgtcatct atgctccaag cagccacaac 2040
aagtatgcag gggagtcatt cccaggaatt tatgtatgtc tgtttgcata tgaaagcaaa 2100
gtggaccctt ccaaggctg gggagaagtg aagagacaga tttatgttgc agccttcaca 2160
gtgcaggcag ctgcagagac tttgagtgaa gtagcc 2196

```

<210> SEQ_ID NO 15

<211> LENGTH: 263

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

```

Met Ala Ser Trp Val Pro Val Val Phe Leu Thr Leu Ser Val Thr Trp
1 5 10 15

```

```

Ile Gly Ala Ala Pro Leu Ile Leu Ser Arg Ile Val Gly Trp Glu
20 25 30

```

```

Cys Glu Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser Arg Gly
35 40 45

```

```

Arg Ala Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val Leu Thr
50 55 60

```

```

Ala Ala His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly Arg His
65 70 75 80

```

```

Ser Leu Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val Ser His
85 90 95

```

```

Ser Phe Pro His Pro Leu Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe
100 105 110

```

```

Leu Arg Pro Gly Asp Asp Ser Ser His Asp Leu Met Leu Leu Arg Leu
115 120 125

```

```

Ser Glu Pro Ala Glu Leu Thr Asp Ala Val Lys Val Met Asp Leu Pro
130 135 140

```

```

Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly
145 150 155 160

```

```

Ser Ile Glu Pro Glu Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val
165 170 175

```

```

Asp Leu His Val Ile Ser Asn Asp Val Cys Ala Gln Val His Pro Gln
180 185 190

```

```

Lys Val Thr Lys Phe Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys
195 200 205

```

```

Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val
210 215 220

```

```

Leu Gln Gly Ile Thr Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu
225 230 235 240

```

```

Arg Pro Ser Leu Tyr Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys
245 250 255

```

```

Asp Thr Ile Val Ala Asn Pro

```

-continued

260

<210> SEQ_ID NO 16
<211> LENGTH: 789
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

atggctactg gggcccccgt tgccttcctc accctgtccg tgacgtggat tggcgctgcg	60
ccccatccatcc tgcctcgat tggggaggc tggagtgcg agaagcatc ccaaccctgg	120
cagggtgttg tggctctcg tggcaggca gtctgcggc gtgttctgg gcaccccaag	180
tgggtcctca cagctgcca ctgcatcagg aacaaaagcg tgatcttgc ggtcggcac	240
agcttgttgc atccctaaga cacaggccag gtatttcagg tcagccacag cttccacac	300
ccgctctacg atatgagcct cctgaagaat cgattcctca ggccaggtga tgactccagc	360
cacgacctca tgctgtccg cctgtcagag cctgccgagc tcacggatgc tgtgaaggc	420
atggacctgc ccacccagga gccagcactg gggaccacct gctacgcctc aggctgggc	480
agcattgaac cagaggagtt cttgacccca aagaaacttc agtgtgtgga cctccatgtt	540
atttccaatg acgtgtgtgc gcaagttcac cctcagaagg tgaccaagtt catgtgtgt	600
gtctggacgtt ggacaggggg caaaaagcacc tgctcgggtt attctggggg cccacttgc	660
tgtatggtg tgcttcaagg tatcacgtca tggggcagtg aaccatgtgc cctgcccga	720
aggccttccc tgtacaccaa ggtgtgcatt accggaagt ggtcaagga caccatgtg	780
gccaacccc	789

<210> SEQ_ID NO 17
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 17

Met Ala Ser Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro			
1	5	10	15

Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Val		
20	25	30

Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Asn		
35	40	45

Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu Asp		
50	55	60

Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr			
65	70	75	80

Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp Ser		
85	90	95

Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu Thr		
100	105	110

Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu Gly		
115	120	125

Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe		
130	135	140

Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His Val Ile Ser Asn			
145	150	155	160

Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe Met Leu	
---	--

US 9,468,672 B2

219**220**

-continued

165	170	175
Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser		
180	185	190
Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp		
195	200	205
Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys		
210	215	220
Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro		
225	230	235
		240

<210> SEQ ID NO 18

<211> LENGTH: 720

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 18

atggctagca ttgtgggagg ctgggagtgc gagaagcatt cccaaacctg gcaggtgctt	60
gtggcctctc gtggcagggc agtctgcggc ggtgttctgg tgcacccca gtgggtcctc	120
acagctgccc actgcatcag gaacaaaagc gtgatcttgc tgggtcggca cagttgttt	180
catcctgaag acacaggcca ggtatttcag gtcagccaca gttcccaaca cccgctctac	240
gatatgagcc tcctgaagaa tcgattcctc aggccagggtg atgactccag ccacgacactc	300
atgctgtctc gcctgtcaga gcctgcccag ctcacggatg ctgtgaaggt catggactg	360
cccacccagg agccagca ggggaccacc tgctacgcct caggctgggg cagcattgaa	420
ccagaggagt tcttgacccc aaagaaaactt cagtgtgtgg acctccatgt tatttcaat	480
gacgtgtgtg cgcaagttca ccctcagaag gtgaccaagt tcatgtgtg tgctggacgc	540
tggacaggggg gcaaaagcac ctgctcggtt gattctgggg gcccacttgt ctgtaatgg	600
gtgcttcaag gtatcacgtc atggggcagt gaaccatgtg ccctgcccga aaggccttcc	660
ctgtacacca aggtggtgca ttaccggaag tggatcaagg acaccatcgt ggccaacccc	720

<210> SEQ ID NO 19

<211> LENGTH: 281

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 19

Met Ala Ser Ala Arg Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val			
1	5	10	15

Leu Ala Gly Gly Phe Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile		
20	25	30

Lys Ser Ser Asn Glu Ala Thr Asn Ile Thr Pro Gly Ile Val Gly Gly		
35	40	45

Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val Leu Val Ala Ser		
50	55	60

Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His Pro Gln Trp Val			
65	70	75	80

Leu Thr Ala Ala His Cys Ile Arg Asn Lys Ser Val Ile Leu Leu Gly		
85	90	95

Arg His Ser Leu Phe His Pro Glu Asp Thr Gly Gln Val Phe Gln Val		
100	105	110

-continued

Ser	His	Ser	Phe	Pro	His	Pro	Leu	Tyr	Asp	Met	Ser	Leu	Leu	Lys	Asn
115						120					125				
Arg	Phe	Leu	Arg	Pro	Gly	Asp	Asp	Ser	Ser	His	Asp	Leu	Met	Leu	Leu
130						135					140				
Arg	Leu	Ser	Glu	Pro	Ala	Glu	Leu	Thr	Asp	Ala	Val	Lys	Val	Met	Asp
145						150					155				160
Leu	Pro	Thr	Gln	Glu	Pro	Ala	Leu	Gly	Thr	Thr	Cys	Tyr	Ala	Ser	Gly
						165					170				175
Trp	Gly	Ser	Ile	Glu	Pro	Glu	Glu	Phe	Leu	Thr	Pro	Lys	Lys	Leu	Gln
						180					185				190
Cys	Val	Asp	Leu	His	Val	Ile	Ser	Asn	Asp	Val	Cys	Ala	Gln	Val	His
						195					200				205
Pro	Gln	Lys	Val	Thr	Lys	Phe	Met	Leu	Cys	Ala	Gly	Arg	Trp	Thr	Gly
						210					215				220
Gly	Lys	Ser	Thr	Cys	Ser	Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Cys	Asn
						225					230				240
Gly	Val	Leu	Gln	Gly	Ile	Thr	Ser	Trp	Gly	Ser	Glu	Pro	Cys	Ala	Leu
						245					250				255
Pro	Glu	Arg	Pro	Ser	Leu	Tyr	Thr	Lys	Val	Val	His	Tyr	Arg	Lys	Trp
						260					265				270
Ile	Lys	Asp	Thr	Ile	Val	Ala	Asn	Pro							
						275					280				

<210> SEQ_ID NO 20
<211> LENGTH: 846
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct
<400> SEQUENCE: 20

atggctagcg	cgcgecgccc	gcgctggctg	tgcgctgggg	cgctggtgct	ggcggtggc	60
ttctttctcc	tcggcttcc	cttcgggtgg	tttataaaat	cctccaatga	agctactaac	120
attactccag	gaatttgtgg	aggctgggag	tgcgagaagc	attcccaacc	ctggcaggtg	180
cttggccct	ctcggtggcag	ggcagttctgc	ggcggtgttc	tggtgacccc	ccagtgggtc	240
ctcacagctg	cccactgcat	caggaacaaa	agcgtgatct	tgctgggtcg	gcacagctg	300
tttcatcctg	aagacacagg	ccaggttattt	caggtcagcc	acagttcccc	acacccgctc	360
tacgatatga	gcctcctgaa	gaatcgattc	ctcaggccag	gtgatgactc	cagccacgac	420
ctcatgtgc	tccgcctgtc	agagcctgcc	gagctcacgg	atgctgtgaa	ggtcatggac	480
ctgcccaccc	aggagccagc	actggggacc	acctgctacg	cctcaggctg	gggcagcatt	540
gaaccagagg	agttcttgac	cccaaagaaa	cttcagttgt	tggaccccca	tgttatttcc	600
aatgacgtgt	gtgcgcaagt	tcaccctcag	aagggtgacca	agttcatgtct	gtgtgtggaa	660
cgctggacag	ggggcaaaag	cacctgctcg	ggtgattctg	ggggcccaact	tgtctgtaat	720
ggtgtgtttc	aaggatcac	gtcatggggc	agtgaaccat	gtgcctgcc	cgaaaggcct	780
tcctgtaca	ccaagggtgt	gcattaccgg	aagtggatca	aggacaccat	cgtggccaac	840
ccctga						846

<210> SEQ_ID NO 21
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

US 9,468,672 B2

223**224**

-continued

<400> SEQUENCE: 21

Met Ala Ser Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala	15
5	10

Leu Gln Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val	30
20	25

Ser Asn Glu Asp Cys Leu Gln Val Glu Asn Cys Thr Gln Leu Gly Glu	45
35	40

Gln Cys Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile	60
50	55

Ser Lys Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln Asp Tyr Tyr	80
65	70

Val Gly Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp Leu Cys Asn Ala	95
85	90

Ser Gly Ala His Ala Leu Gln Pro Ala Ala Ala Ile Leu Ala Leu Leu	110
100	105

Pro Ala Leu Gly Leu Leu Trp Gly Pro Gly Gln Leu	125
115	120

<210> SEQ ID NO 22

<211> LENGTH: 375

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

atggctagca aggctgtgct gcttgcctg ttgatggcag gcttggccct gcagccaggc 60

actgcccgtc tgcgtactc ctgcaaagcc caggtgagca acgaggactg cctgcaggcg 120

gagaactgca cccagctggg ggagcagtgc tggaccgcgc gcatccgcgc agttggcctc 180

ctgaccgtca tcagcaaagg ctgcagcttg aactgcgtgg atgactcaca ggactactac 240

gtggccaaga agaacatcac gtgctgtgac accgacttgt gcaacgccag cggggccat 300

gccctgcagc cggctgccgc catccttgcg ctgctccctg cactcggcct gctgtctgg 360

ggaccgggcc agcta 375

<210> SEQ ID NO 23

<211> LENGTH: 5964

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 23

ggcgtaatgc tctgcccagt ttacaaccaa ttaaccaatt ctgatttagaa aaactcatcg 60

agcatcaaat gaaaactgca tttattcata tcaggattat caataccata tttttaaaa 120

agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc 180

tggtatcggt ctgcgttcc gactcgtcca acatcaatac aacctattaa tttccctcg 240

tcaaaaataa gtttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat 300

ggcaaaaact tatgcatttc tttccagact tggtaacacg gccagccatt acgctcgta 360

tcaaaaatcac tcgcatcaac caaacgtta ttcattcgtg attgcgcctg agcgagacga 420

aatacgcgt cgtgttaaa aggacaatta caaacaggaa tcaaattgca cccggcgcagg 480

aacactgcca ggcgttcaac aatatttca cctgaatcg gatatttttc taataacctgg 540

aatgctgttt tccccggat cgcagtggtg agtaaccatg catcatcagg agtacggata 600

aaatgcttga tggtcggaag aggataaat tccgtcagcc agtttagtct gaccatctca 660

-continued

tctgttaacat cattggcaac gctacccttg ccatgtttca gaaacaactc tggcgcatcg	720
ggcttccat acaatcgata gattgtcgca cctgattgcc cgacattatc gcgagccat	780
ttataccat ataaatcagc atccatgttga aatattaatc ggggcctcga gcaagacgtt	840
tcccgttgaat tatggctcat aacaccctt gtattactgt ttatgtaaac agacaggctcg	900
acaatattgg ctattggcca ttgcatacgt tgtatctata tcataatatg tacatttata	960
ttggctcatg tccaatatga ccgcctatgtt gacattgatt attgactagt tattaatagt	1020
aatcaattac ggggtcatta gttcatagcc catatatggaa gttccgcgtt acataactta	1080
cggtaatgg cccgcctggc tgaccgccta acgacccccc cccattgacg tcaataatga	1140
cgtatgttcc catagtaacg ccaataggga ctttccatttgc acgtcaatgg gtggagttt	1200
tacggtaaac tgcccacttg gcagtagatc aagtgtatca tatgccaatgt ccgcggcccta	1260
ttgacgtcaa tgacggtaaa tggccgcctt ggcattatgc ccagtagatc accttacggg	1320
actttccatc ttggcagttac atctacgtat tagtcatcgc tattaccatg gtgtgcgggt	1380
tttggcagta caccaatggg cgtggatagc gggttgactc acggggattt ccaagtctcc	1440
accccatgtg cgtcaatggg agtttggttt ggcacccaaa tcaacgggac tttccaaaat	1500
gtcgtataaa ccccgccccg ttgacgcaaa tgggcggtag ggcgtgtacgg tggggaggtct	1560
atataagcag agctcggtta gtacccgcgc agatcgctg gagacgcac ccacgctgtt	1620
ttgacctcca tagaagacac cgggaccgtat ccagccctcg cggccgggaa cgggtcatttgc	1680
gaacgeggat tccccgtgcc aagagtgtactt caccgtccgg atctcagccaa gcaggatgt	1740
actctccagg gtgggcctgg ctccccctgtt caagacttca gggatttgag ggacgctgt	1800
ggctcttc ttagatgtac ctccatgttgc cctcaaccctt gactatcttc caggtcagga	1860
tcccagagtc aggggtctgt atttccctgc tgggtggctcc agttcaggaa cagtaaaccc	1920
tgctccgaat attgcgttcc acatctcgcc aatctcccg aggactgggg accctgtgac	1980
gaacatggct agcgcgcgc gccccgcgtg gctgtgcgtt gggcgctgg tgctggccgg	2040
tggctttt ctccctggct tcccttccgg gtgggttata aaatccctca atgaagctac	2100
taacattact ccaaagcata atatgaaacg atttttggat gaattgaaag ctgagaacat	2160
caagaagttc ttatataatt ttacacatg accacatttgc cagggaaacag aacaaaactt	2220
ttagcttgca aagcaaatttcc aatcccagtg gaaagaattt ggcctggatt ctgtttagt	2280
ggcacattat gatgtcctgt tgccttaccc aaataagact catcccaactt acatctcaat	2340
aattaatgaa gatggaaatg agatttcaaa cacatcatta tttgaaccac tccctccagg	2400
atatgaaaat gtttcggata ttgttaccacc tttcagtgttgc ttctcttc aaggaatgcc	2460
agagggcgtat ctagtgtatg ttaactatgc acgaactgaa gacttcttta aattggaaacg	2520
ggacatgaaa atcaattgtctt ctggggaaat tgtaattgc agatatgggaa aagtttccag	2580
aggaaataag gttaaaaatg cccagctggc agggggccaaa ggagtcatttcc tctactccgaa	2640
ccctgtgtac tactttgtcc ctgggggttgc gtcctatcca gatgggttgcg atcttcgtt	2700
aggtgggtgtc cagcgtggaa atatcctaaa tctgaatgttgc agggaggacc ctctcacacc	2760
aggttaccca gcaaatgaaat atgcttatag gctgttgc gcaaggccgtt ttgggtctcc	2820
aagtattccctt gttcatccaa ttggataacta tgcgtacacg aagcttccatg aaaaaatggg	2880
tggctcagca ccaccagata gcagctggag aggaagtc tccatgcctt acaatgttgg	2940
acctggctttt actggaaactt tttctacaca aaaagtcaag atgcacatcc actctaccaa	3000

-continued

tgaagtgaca agaatttaca atgtgatagg tactctcaga ggagcagtgg aaccagacag	3060
atatgtcatt ctggggaggc accggggactc atgggtgttt ggtggatattg accctcagag	3120
tggagcagct gttgttcatg aaattgttag gagctttgaa acactgaaaa aggaagggtg	3180
gagacctaga agaacaattt tggttgcag ctgggatgca gaagaatttg gtcttctgg	3240
ttctactgag tgggcagagg agaattcaag actccttcaa gagcgtggcg tggcttat	3300
taatgtcag tcatctatag aaggaaacta cactctgaga gttgatttgc caccgctgat	3360
gtacagctt g tacacaacc taacaaaaga gctgaaaagc cctgatgaa gctttgaagg	3420
caaattctt tatgaaagtt ggactaaaaa aagtccctcc ccagagttca gtggcatgcc	3480
caggataagc aaattggat ctggaaatga ttttggatgt ttcttccaac gacttggaaat	3540
tgcttcaggc agagcacggc atactaaaaa ttggaaaca aacaaattca gcggctatcc	3600
actgtatcac agtgtctatg aaacatatga gttggggaa aagttttatg atccaatgtt	3660
taaatatcac ctcactgtgg cccaggttcg aggagggatg gtgtttgagc tggccaattc	3720
catagtgctc ccttttgcatt gtcgagatata tgctgtatgt ttaagaaagt atgctgacaa	3780
aatctacagt atttctatga aacatccaca gggaaatgaa acatacagtg tatcatttgaa	3840
ttcactttt tctgcagtaa agaattttac agaaatttgc tccaagttca gtgagagact	3900
ccaggacttt gacaaaagca acccaatagt attaagaatg atgaatgatc aactcatgtt	3960
tctggaaaga gcatttattt atccatttagt gttaccagac aggcctttt ataggcatgt	4020
catctatgtc ccaaggcagcc acaacaagta tgcagggggatc tcattccag gaatttatgaa	4080
tgctctgttt gatattgaaa gcaaaatgtt cccttccaag gcctggggag aagtgaagag	4140
acagatttt gttgcagcct tcacagtgc ggcagctgca gagactttga gtgaagtagc	4200
ctaaagatct gggccctaacc aaaacaaaaa gatggggta ttccctaaac ttcatgggtt	4260
acgttaattgg aagttggggg acattgccac aagatcatat tgtacaaaag atcaaacact	4320
gttttagaaa acttccgtta aacaggccta ttgattggaa agtatgtcaa aggattgtgg	4380
gtctttggg ctttgcgtct ccatttacac aatgtggata tctgcctta atgccttgc	4440
atgcgtat acaagctaaa caggcttca ctttctcgcc aacttacaag gccttctaa	4500
gtaaacagta catgaacctt taccgggtt ctcggcaacgc gcctggctcg tgccaagtgt	4560
ttgctgcgc aacccccact ggctggggct tggccatagg ccatcagcgc atgcgtggaa	4620
ccttgcgtgc tccctcgcc atccatactg cgaaactcct agccgcttgt tttgcgtgc	4680
gcgggtctgg agcaaaagctc ataggaactg acaattctgt cgtcctctcg cggaaatata	4740
catcgttcg atctacgtat gatcttttc cctctgccaa aaattatggg gacatcatgaa	4800
agcccccttgc gcatctgact tctggctaat aaaggaaattt tattttcatt gcaatagtgt	4860
gttggaaattt tttgtgtctc tcactcgaa ggaattctgc attaatgaaat cggccaaacgc	4920
ggggggagag ggggttgcg tattggggc tttccgtttt cctcgtcac tgactcgctg	4980
cgctcggtcg ttccggctgcg gcggcggtt tcagctact caaaggcggt aatacggtt	5040
tccacagaat cagggataa cgcaggaaag aacatgttag caaaaggcca gcaaaaggcc	5100
aggaaccgtt aaaaaggccgc gttgctggcg ttttccata ggctccgccc ccctgacgag	5160
catcacaaaa atcgacgctc aagtcagagg tggcgaaacc cgacaggact ataaagatac	5220
caggcggttc cccctggaaat ctccctcgat cgtctctcg tttccgtttt cccgttacc	5280
ggataacctgt ccgccttttcccttccggaa agcggtggcg tttctcatag ctcacgctgt	5340
aggtatctca gttcggtgtt ggtcggtcg tccaaatgtt gctgtgtgc cgaaccccccc	5400

-continued

gttcagcccg accgctgcgc cttatccggtaactatcgcttgagtccaa cccggtaaga	5460
cacgacttat cgccactggc agcagccact ggtaacaggattagcagagc gaggtatgtat	5520
ggcggtgcta cagagttctt gaagtgggtgg cctaactacg gctacactag aagaacagta	5580
tttggtatct gcgcctctgtctt gaagccaggta accttcggaa aaagagttgg tagctttgtat	5640
tccggcaaac aaaccaccgc tggtagccgggt ggttttttttggtttcaagca gcagattacg	5700
cgcagaaaaa aaggatctca agaagatctt ttgatctttt ctacggggtc tgacgctcag	5760
tggAACGAAA actcacgtta agggatttttggcatgagat tatcaaaaag gattttacc	5820
tagatccccc taaattaaaa atgaagttttt aaatcaatctt aaagtataata tgagtaaact	5880
tggctgaca gttaccaatgtt cttaatcagt gaggcaccta tctcagcgat ctgtctattt	5940
cgttcatcca tagttgcctg actc	5964

<210> SEQ ID NO 24
<211> LENGTH: 4122
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 24	
ggcgtaatgc tctgccaggtttacaaccaa ttaaccaatttctgatttagaa aaactcatcg	60
agcatcaat gaaaactgcaat tttattcata tcaggattat caataccata tttttgaaaa	120
agccgttctt gtaatgaagg agaaaaactca ccggggcaggatccataggat ggcaagatcc	180
tggtatcggtt ctgcgattcc gactcgatca acatcaatac aacctattaa tttccctcg	240
tcaaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatccggtgagaat	300
ggcaaaagct tatgcatttc tttccagact tttcaacag gccagccattt acgctcgatca	360
tcaaaatcac tcgcatcaac caaacgtta ttcatcgat attgcgcctg agcggagacga	420
aatacgcgtatcgatccatggacaat taaaacaggaa tcaaatgcggggcagg	480
aacactgcca ggcgcataac aatattttca cctgaatcgat gatattcttc taatacctgg	540
aatgctgttt tccccggat cgcagggttg agtaaccatcgatcatcagg agtaeggata	600
aaatgcttgc tggtcggaaagg aggatcaat tccgtcagcc agtttagtctt gaccatctca	660
tctgtacat cattggcaac gctaccttgc ccatgttca gaaacaactc tggcgatcg	720
ggcttccat acaatcgata gattgtcgat cctgattggcccgacattatc gcgagccat	780
ttatacccat ataaatcagc atccatgttg gaatttataatc gggccctcgatcaagacgtt	840
tcccggttgcataatgttgc aacacccctt gtattactgtt ttagtgcatac agacaggatcg	900
acaatattgg ctattggcca ttgcataatgttgc tttatctataatc tacattata	960
ttggctcatg tccaaatgttgc cccatgttgc gacattgttatttgcactatgttataatgtt	1020
aatcaattac ggggtcattatgttgc gttcatagcc catatatggat tttcccgatcgatcaactt	1080
cggtaatgg cccgcgttgc tgaccggccaa acgaccccccggccatgttgc tcaataatgtt	1140
cgtatgttcc cttatgttgc ccaataggatgc ctttccatgttgc tttatgttgc gttggatgtt	1200
tacggtaaac tggccacttgc gcaatgttgc aatgttgc tttatgttgc tttatgttgc tttatgttgc	1260
ttgacgttcaatgttgc tggccatgttgc ggcattatgttgc ccaatgttgc accttacggatgtt	1320
actttccatgttgc tttatgttgc tttatgttgc tttatgttgc tttatgttgc tttatgttgc tttatgttgc	1380
tttggcagttcaatgttgc cgtggatgttgc ggtttggacttgc acggggatgttgc ccaatgttgc	1440

-continued

accccatggta cgtcaatggg agtttgggtt ggcacccaaa tcaacgggac tttccaaaat 1500
gtcgtaataa ccccgecccc ttgacgc当地 tggcggtag gegtgtacgg tggggaggct 1560
atataagcag agctcggtt gtgaaaccgtc agatcgctg gagacccat ccacgcgtt 1620
ttgaccccca tagaagacac cgggaccgt ccagcctcg eggccggaa cggtcattg 1680
gaacgcggat tccccgtgcc aagagtact caccgtccgg atctcagcaa gcaggatgt 1740
actctccagg gtggccctgg cttecccagt caagactcca gggattttag ggacgcgttg 1800
ggcttcttc ttacatgtac cttttgctt cctcaaccct gactatctt caggtcagg 1860
tcccagagtc aggggcttgtt atttccctgc tggtggctcc agttcaggaa cagtaaaccc 1920
tgctccgaat attgcctctc acatctcgta aatctcccg aggactgggg accctgtgac 1980
gaacatggct agcaaggctg tgctgcttgc cctgtttagt gcaggcttgg ccctgcagcc 2040
aggcactgccc ctgctgtgt actctcgaa agcccaggta agcaacgagg actgcctgca 2100
ggtggagaac tgcacccagc tgggggagca gtgtggacc ggcgcgatcc ggcgcgttgg 2160
cctcctgacc gtcatcagca aaggctgcag cttgaactgc gtggatgact cacaggacta 2220
ctacgtggcc aagaagaaca tcacgtgtg tgacaccgc ttgtgcaacg ccagcggggc 2280
ccatgcccctg cagccggctg cgcgcattct tgcgtgtgtc cctgcactcg gcctgtgt 2340
ctggggaccgc ggccagctat agagatctgg gcccataacaa aacaaaaaga tggggttatt 2400
ccctaaactt catgggttac gtaattggaa gttggggac attgcaccaa gatcatattg 2460
tacaaaagat caaacactgt ttttagaaaac ttctgtaaa caggccattt gattggaaag 2520
tatgtcaaaag gattgtgggt cttegggtt ttgtgtctcc atttacacaa tgtggatattc 2580
ctgccttaat gcctttgtat gcatgtatac aagctaaaca ggcttctact ttctcgccaa 2640
cttacaaggc ctttctaagt aaacagtaca tgaacctta ccccggtct cggcaacggc 2700
ctggctgtgtt ccaagtgtt gctgacgc当地 ccccaactgg ctgggggtt gccataggcc 2760
atcagegc当地 gcgtggaaacc ttgtggctc ctgc当地ccat cctactgc当地 gaactcttag 2820
ccgtgtttt tgctcgagc cggcttggag caaagctcat aggaactgac aatttgc当地 2880
tctctcgcc gaaatataca tcgtttgc当地 ctacgtatga tcttttccc tctgcaaaaa 2940
attatggggc catcatgaag ccccttgagc atctgacttc tggctataaa aggaaattta 3000
ttttcattgc aatagtgtgt tggattttt tggatctctc actcgaaagg aatttgc当地 3060
taatgaatcg gccaacgc当地 ggggagaggg ggttgc当地 ttggggctcc ttccgcttcc 3120
tcgctcactg actcgctgctc ctgc当地tgc当地 cggctgccc gagcggatcc agctcactca 3180
aaggcggtaa tacgggttac cacagaatca gggataacg cagggaaagaa catgtgagca 3240
aaaggccagc aaaaggccag gaaccgtaaa aaggccgc当地 tgctggctt ttccatagg 3300
ctccggcccc ctgacgagca tcacaaaaat cgacgc当地 gtcagaggta ggc当地acccgg 3360
acaggactat aaagatacca ggc当地ttccc cctggaaagct ccctcgctc ctctctgtt 3420
ccgaccctgc cgcttaccgg atacctgtcc gc当地ttctcc ctgc当地ggaaag cggtggctt 3480
tctctatgact caccgtgttagt gtatctcactg tggatctgg tggatctctc caagctggcc 3540
tggatgtgc当地 aaccccccgt tcaaccgc当地 cgtgc当地ct tatccggtaa ctatctgtt 3600
gagttcaacc cggtaagaca cggacttacg ccactggc当地 cggccactgg taacaggatt 3660
agcagagc当地 ggtatgttagg cggctgctaca gagttcttga agtggggcc taactacggc 3720
tacactagaa gaacagtatt tggatctgc gctotgtga agccagttac ctccggaaaa 3780
agagttggta gctcttgc当地 cggcaaccaa accaccgtc gtagcggcttgg ttttttgg 3840

-continued

tgcaaggcgc agattacgcg cagaaaaaaa gnatctcaag aagatcctt gatctttct	3900
acggggctcg acgctcagtg gaacgaaaac tcacgttaag ggattttgt catgagatta	3960
tcaaaaagga tttcaccta gatccttta aattaaaaat gaagttttaa atcaatctaa	4020
agtatatatg agtaaacctg gtctgacagt taccaatgct taatcagtga ggcacctatc	4080
tcagcgatct gtctatccg ttcatccata gttgcctgac tc	4122

<210> SEQ ID NO 25

<211> LENGTH: 4467

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 25

ggcgtaatgc tctgccagtg ttacaaccaa ttaaccaatt ctgatttagaa aaactcatcg	60
agcatcaaat gaaactgcaa tttattcata tcaggattat caataccata tttttgaaaa	120
agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgattcc gactcgatca acatcaatac aacctattaa tttcccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaaact tatgcatttc tttccagact tggtaacacg gccageccatt acgctcgatca	360
tcaaaaatcac tcgcatcaac caaaccgtt ttcattcgat attgcgcctg agcgagacga	420
aatacgcgat cgctgttaaa aggacaatta caaacaggaa tcaaatgca cggcggcagg	480
aacactgcca gcgcataac aatattttca cctgaatcag gatattcttc taatacctgg	540
aatgctgttt tccccgggat cgcagtgggt agtaaccatg catcatcagg agtaeggata	600
aaatgcttga tggtcgaaagg aggataaat tccgtcagcc agtttagtct gaccatctca	660
tctgttacat cattggcaac gctacccttg ccatgttca gaaacaactc tggcgatcg	720
ggcttccat acaatcgata gattgtcgaa cctgattgccc cgacattatc gcgagccat	780
ttatacccat ataaatcagc atccatgttg gaatttaatc gggccctcgaa gcaagacgtt	840
tcccggttata tgggttcat aacaccctt gtattactgt ttatgttacg agacaggtcg	900
acaatattgg ctattggca ttgcataatgt tggatctata tcataatatg tacattata	960
ttggctcatg tccaaatatga ccgcattgtt gacattgatt attgactagt tattatagt	1020
aatcaattac ggggttattt gttcatatgc catatatggaa gttccgcgtt acataactta	1080
cggtaaatgg cccgcctggc tgaccggcca acgacccccc cccattgacg tcaataatga	1140
cgtatgttcc catagtaacg ccaataggaa cttccattt acgtcaatgg gtggagtatt	1200
tacggtaaac tgcccaatgg gcaatcgttca aagtgtatca tatgcaatgtt ccggccctta	1260
ttgacgttca tgacgtttaa tggcccgctt ggcattatgc ccagtacatg accttacggg	1320
actttccatc ttggcgttac atctacgtat tagtcatcg tattaccatg gtgtatgcgggt	1380
tttggcgttca caccatggg cgtggatagc ggatggactc acggggattt ccaatgttcc	1440
accccatgtt ggtcaatggg agtttgggtt ggcacccaaa tcaacgggac tttccaaaat	1500
gtcgttataa ccccgccccg ttgacgttcaaa tggccgttag gcaatgttccgg tggggaggct	1560
atataagcag agctcgatca gtgtacccgtc agatcgatcg gacacgcccattt ccacgttcc	1620
ttgacccatca tagaagacac cgggaccat ccaggctccg cggccggaa cgggttcc	1680
gaacgcggat tccccgttca aagatgttca caccgttccgg atctcagcaaa gcaggtatgt	1740

-continued

actctccagg gtgggectgg cttccccagt caagactcca gggattttag ggacgtgtg
ggcttctc ttacatgtac ctttgcctg cctcaacct gactatctc caggtcaggaa
tcccagagt aggggctgt attttcctgc tggggctcc agttcaggaa cagtaaaccc
tgctccgaat attgcctctc acatctcgac aatctccgcg aggactgggg accctgtgac
gaacatggc agcattgtgg gaggctggg gtgcgagaag cattccaaac cctggcaggt
gcttggcc tctcggtggca gggcagtcg cggcgggtt ctgggtcacc cccagtggt
cctcacagct gcccactgca tcaggaacaa aagcgtgatc ttgctgggtc ggcacagctt
gtttcatctt gaagacacag gccaggtatt tcaggtcagc cacagttcc cacaccggct
ctacgatatg agcctccatg agaatcgatt cctcaggcca ggtgtact ccagccacga
cctcatgtc ctccgcctgt cagagcctgc cgagctcagc gtgtgtgtga aggtcatgg
cctgcccacc caggagccag cactggggac cacctgtac gcctcaggct gggcagcat
tgaaccagag gagttcttgc ccccaaagaa acttcagtgt gtggacctcc atgttatttc
caatgacgtg tgtgcgcaag ttcaccctca gaaggtgacc aagttcatgc tgtgtgttgg
acgctggaca gggggcaaaa gcacccgttc ggggtattt gggggccac ttgtgttgg
tgggtgttgc caaggtatca cgtcatgggg cagtgaacca tgtgcctgc ccgaaaggcc
ttccctgtac accaagggtt tgccattaccg gaagtggatc aaggacacca tctgtggccaa
cccoctgaaga tctggccctt aacaaaacaa aaagatgggg ttattcccta aacttcatgg
gttacgtata tggaaagttgg gggacattgc cacaagatca tattgtacaa aagatcaaac
actgttttag aaaacttctt gtaaacaggc ctattgattt gaaagtatgt caaaggattt
tgggtctttt gggctttgtt gctccattt cacaatgtgg atatctggcc ttaatgcctt
tgtatgcatttatacaagttt aacaggctt tcactttctc gccaacttac aaggccttcc
taagtaaaca gtacatgaac cttaaaaaaccc ttgtcggtt acggccgtt ctgtgttgg
tgggtgttgc cgcaacccccc actggctggg gcttggccat aggccatcag cgcatgcgt
gaacctttgtt ggctccctgtt ccgttccata ctggggactt cttttttgtt tggttgc
cgagccgggtt tggagccaaat ctcattttttt ctgacaattt tgggtgttgc tggggaaat
atacategtt tggatctacg tatgtatctt ttccctgttgc caaaaattttt ggggacatca
tgaagccctt tgagcatctt acttctggct aataaaggaa atttattttt attgttatag
tgtgttggaa ttttttgcgtt ctctacttcg gaaggaattt tggatattatg aatcgccaa
cgccggggaa gaggccgtttt cggttattttt ccgttcttcg ctccctcgct cactgactcg
ctggcgttgc tggatctggctt gggccgggtt gtatcgatcc actcaaaaggc ggttacacgg
ttatccacag aatcaggggaa taacgcaggaa aagaacatgtt gggcaaaagg ccagcaaaag
gccaggaaacc gtaaaaaaggc cgcgttgcgtt gctttttcc ataggctccg ccccccgttgc
gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa accccgacagg actataaaga
taccaggcgtt ttccctgttgc aagctccctc gtgcgttgc ctgttccgtt cctggccgtt
accggatacc tggatctggctt tctcccttcg ggaagcggtt cgcttctca tagctcactcg
tgttaggtatc tcaatgttgcgtt gtaggttcgtt cgcttccaaacg tgggtgttgc gacacaaacc
cccggttgcgtt ccgttccatcc ggttacttac gtttttttttgcgtt ccacccggat
agacacgact tatcgccactt ggcagcggcc actggtaaca ggatttagcag agcgaggat
gtggccgttgc tcaatgttgcgtt gtaggttcgtt ccgttccatcc acggcttacac tagaagaaca
gtatgttgcgtt tctcggttgcgtt gtttttttttgcgtt ccacccggat 4140

-continued

tgatccggca aacaaaccac cgctggtagc ggtggtttt ttgttgcaa gcagcagatt	4200
acgcgcagaa aaaaaggatc tcaagaagat ccttgcgtct ttctacggg gtctgacgct	4260
cagtggAACG aaaactcacg ttaaggatt ttggcatga gattatcaa aaggatctc	4320
acctagatcc tttaaattaa aaaatgaagt tttaaatcaa tctaaagtat atatgagtaa	4380
acttggtctg acagttacca atgcttaatc agtgaggcac ctatctcagc gatctgtcta	4440
tttcgttcat ccatagttgc ctgactc	4467

<210> SEQ ID NO 26
<211> LENGTH: 7563
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 26	
gaattctgca ttaatgaatc ggccaacgcg cggggagagg cggtttgctt attggggcgt	60
cttccggttc otcgctcaact gactcgctgc gctcggtcggtt cggctgcgg cgagcggtat	120
cagctcaactc aaaggccgttatacgggtt ccacagaatc aggggataac gcagggaaaga	180
acatgtgagc aaaaggccag caaaaggcca ggaaccgtaa aaaggccgcg ttgtggcgt	240
ttttccatag gtcggcccc cctgacgagc atcacaaaaaa tcgacgctca agtcagaggt	300
ggcgaaaccc gacaggacta taaagataacc aggcgtttcc coctggaagc tccctcgtc	360
gtctctgtt tccgaccctg ccgttaccg gatacctgtc cgccttctc ccttcggaa	420
gctggcgct ttctatagc tcacgctgtt ggtatctcgat ttccgtgttag gtcgttcgct	480
ccaagctggg ctgtgtgcac gaacccccc ttcagcccgaa cccgtcgcc ttatccggta	540
actatcgctc tgagtccaaac ccggtaagac acgacttatac gcaactggca gcagccactg	600
gttaacaggat tagcagagcg aggtatgttag gccgtgtac agagttcttg aagtggggc	660
ctaactacgg ctacactaga agaacatgtt tttgttatctg cgctctgctg aagccaggta	720
ccttcggaaa aagagtttgtt agtcttgcgtt ccggcaaaaca aaccaccgct ggttagcggt	780
gttttttgtt ttgcaagcag cagattacgc gcagaaaaaa aggatctcaa gaagatcctt	840
tgatcttttc tacggggctc gacgctcgtt ggaacgaaaa ctcacgtttaa gggattttgg	900
tcatgagatt atcaaaaagg atcttcaccc agatcctttt aaattaaaaa tgaagttta	960
aatcaatcta aagtatatat gagtaaactt ggtctgacag ttaccaatgc ttaatcgtt	1020
aggcacctat ctcagcgatc tgtcttatttc gttcatccat agttgcctga ctcggcgtaa	1080
tgctctgcca gtgttacaac caattaacca attctgatta gaaaaactca tcgagcatca	1140
aatgaaaactg caatttattc atatcaggat tatcaataacc atattttga aaaagccgtt	1200
tctgtatga aggagaaaaac tcaccggggc agttccatag gatggcaaga tcctggatc	1260
ggtctcgat tccgactcgtt ccaacatcaa tacaacccat taattttccc tcgtcaaaaa	1320
taaggttatc aagtggaaaaa tcaccatgag tgacgactga atccggtgag aatggcaaaa	1380
gtttatgcattt ttctttccag acttgcgtt caggccagcc attacgcgtc tcatcaaaat	1440
cactcgcatc aaccaaaccg ttattcattc gtgattgcgc ctgagcgaga cgaaatacgc	1500
gatcgctgtt aaaaggacaa ttacaacag gaatcaaatg caacccggcgc aggaacactg	1560
ccagcgcatc aacaatattt tcacccatc caggatattc ttctaatacc tggaatgcgt	1620
ttttccgggg gatcgactgt gttagtaacc atgcatcatc aggagtgacgg ataaaaatgct	1680

-continued

tgatggcgcg aagaggcata aattccgtca gccagtttag tctgaccatc tcatctgtaa 1740
catcattggc aacgcgtactt ttgccatgtt tcagaaacaa ctctggcgca tcggggttcc 1800
cataacaatcg atagattgtc gcacccgtatt gcccgcatt atcgcgagcc catttataacc 1860
catataaaatc agcatccatg ttgaaattta atcgcggcct cgagcaagac gtttccgtt 1920
gaatatggct cataaacaccc cttgtattac tgtttatgtt agcagacagg gtaccaatct 1980
tccgagttag agacacaaaa aattccaaca cactattgca atgaaaataa atttccttta 2040
tttagccagaa gtcagatgct caaggggctt catgtatgtcc ccataatttt tggcagaggg 2100
aaaaagatca tacgttagatc gaaacgatgt atattccgc gagaggacga cagaattgtc 2160
agttccatag agctttgctc cagaccggct gcgagcaaaa caagcggcta ggagttccgc 2220
agtagatggatc ggcagaggag ccacaaaggt tccacgcgtt cgctgtatggc ctatggccaa 2280
gccccagcca gtgggggttg cgtagcaaa cacttggcac agaccaggcc gttggcgagc 2340
aacggggtaa aggttcatgt actgttact tagaaaggcc ttgtaagttt gcgagaaagt 2400
gaaagcctgt ttagcttgta tacatgcata caaaggcatt aaggcaggat atccacattt 2460
tgttaatggta gcagcaaaagc cccaaagacc cacaatccctt tgacataactt tccaatcaat 2520
aggcctgtttt acaggaagtt ttctaaaaca gtgtttgatc tttgtacaa tatgtatctt 2580
tggcaatgtc ccccaacttc caattacgtt acccatgtt aacccatctt aacccatct 2640
ttttgtttt ttagggccca gatctttagg ctacttcactt caaagtctct gcagctgcct 2700
gcactgtgaa ggctgcaca taaatctgtc tcttcacttc tccccaggcc ttgaaagggt 2760
ccactttgtt ttcaatataca aacagagcat cataaattcc tggaatgtac tccccgtcat 2820
acttgttggt gctgcttggc gcatagatgtt catgcctata aaaaggtctg tctggtaacc 2880
ctaattggatc aataaatgtt cttccatggaa acatgagttt atcatttcattt attcttata 2940
ctattgggtt gctttgtca aagtcttggc gtctctactt gaacttggaa gcaatttctg 3000
taaaatttctt tactgcagaa aaaagtgaat caaatgatac actgtatgtc ttcatttctt 3060
gtggatgtttt catagaataa ctgttagattt tgcacgttca cttttttttt actacagcat 3120
aatctcgaca atcaaaaaggc agcactatgg aattggccag ctcaaacacc atccctctc 3180
gaacctgggc cacagtgggg tgatattttt acattggatc ataaaactttt tccaccaact 3240
catatgtttt atagacactg tgatcactgt gatagccgtt gaatttgggtt gtttccat 3300
tttttagtata ccgtgtctg cctgaagca ttccaagtttgc ttggaagaac acctctaaat 3360
cattttccaga tcccaattttt cttatcctgg gcatgccactt gaactctggg gaaggacttt 3420
tttttagtccca actttccatca aagagattgtc cttcaaaagcc ttcatcagggtt cttttcagct 3480
ctttttgttag gttgtgtacc aagctgtaca tcagcgggtt acaatcaactt ctcagagtgt 3540
agtttccatcc tataatgttgc tgatcatttttataagccac gcccacgttct tgaagggtt 3600
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 3660
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 3720
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 3780
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 3840
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 3900
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 3960
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 4020
tttgcatttttccatcc tctgtccac tcagtagaaac caagaagacc aaatttttctt gcatccccagg 4080

-continued

tataaggata ttcatttgct gggtaacctg gtgtgagagg gtctcctgca ccattcagat	4140
tttagatatt tccacgctgg acaccaccc caggaagatt ccaaccatct ggataggact	4200
tcaccccagg agcaaagtag tcagcagggt cgaggtagag aatgactcct ttggccctg	4260
ccagctgggc attttaacc ttatccctc taaaactttt cccatatctg gcaattacaa	4320
tttcccaga gcaattgatt ttcatgtccc gttccaattt aaagaagtct tcagttcg	4380
catagttaac atacactaga tcgcctctg gcattccctg aggagagaaa gcactgaaag	4440
gtggtacaat atccgaaaca tttcatatc ctggaggagg tggttcaaattt aatgtgtgt	4500
tgaaaatctc atttccatct tcattaatta tttagatgtat gttggatgtat gtcattttt	4560
ggtaggacaa caggacatca taatgtgcca gctcaacaga atccaggcca aattttcc	4620
actgggattt aatttgcattt gcaagctgaa agttttgttc tggttctgtt aaatgtggta	4680
tctgtgtaaa attatataag aacttcttgc tggttctcagg tttcaatttca tccaaaaatg	4740
ctttcatatt atgctttggaa gtaatgttag tagtttcattt ggaggattt ataaaccacc	4800
cgaagaggaa gccgaggaga aagaagccac ccgcgcgcac cagcgcgcgc ggcacagcc	4860
agcgccccgc ggcgcgccta gccatgttcg tcacagggtt cccagtttc gcccggattt	4920
acgagatgtg agaggcaata ttccggacgag ggtttactgt tctgtactg gagccacag	4980
caggaaaata cagacccctg actctggat cctgacctgg aagatagtca gggttgggc	5040
aagcaaaagg tacatgttaag agaagagccc acagcgtccc tcaaatttcc gtagtcttgc	5100
ctggggaaagc caggeccacc ctggagagta catacctgct tgctgagatc cggacgggt	5160
gtcacttttgc acacggggaa tccgcgttcc aatgcacgt tcccgccgc ggaggcttgg	5220
tegggtcccg tgcattttat ggaggtaaaa acagcgtggaa tggcgtctcc aggcgtatgt	5280
acgggttactt aaacgagctc tgcttatata gacctccac cgtacacgcc taccgcct	5340
ttgcgttcaac ggggggggt tattacgaca ttttggaaag tcccggtat tttgggtgtc	5400
gacctgcagg gtaccaatat tggctattgg ccattgcata cgttgtatct atatcataat	5460
atgtacattt atattggctc atgtccaata tgaccgcattt gttgacatgtt attattgtact	5520
agtttataat agtaatcaat tacggggtca ttagttcata gcccataat gtagtccgc	5580
gttacataac ttacggtaaa tggeccgcgtt ggctgacccgc ccaacgcacc cggccattt	5640
acgtcaataa tgacgtatgt tcccatagta acgccaatag ggactttcca ttgacgttca	5700
tgggtggagt atttacggta aactgcccac ttggcgtatgt atcaagtgttca tcatatgcca	5760
agtccgcgcgc ctattgacgt caatgacgtt aaatggcccg cctggcatat tggccgtatgt	5820
atgaccttac gggactttcc tacttggcag tacatctacg tatttgtcat cgcttattacc	5880
atgggtatgc gttttggca gtacaccaat gggcgtggat agcgggttgc ctcacgggaa	5940
tttccaatgc tccacccat tgacgtcaat gggagtttgc tttggcacca aaatcaacgg	6000
gactttccaa aatgtcgtaa taacccgc cccgttgcgc aaatggccgg taggcgtgtt	6060
cgggtggagg tctatataag cagagctgtt ttagtgaacc gtcagatcgc ctggagacgc	6120
catccacgt gttttgcattt ccataagaaga caccggacc gatccgcctt cccggccgg	6180
gaacgggtca ttggAACGCG gattccccgtt gccaaggatgtt actcaccgtc cggatctcag	6240
caaggcggta tgcgtacttcc aggggtggcc tggctttccc agtcaagactt ccagggattt	6300
gagggacgtt gtgggtctt ctcttacatg taccttttgc ttgcctcaac cctgtactatc	6360
ttccaggtaa ggatcccaga gtcagggttc tgcattttcc tgcgtggc tccagttcag	6420

-continued

gaacagtaaa ccctgctccg aatattgcct ctcacatctc gtcaatctcc gcgaggactg	6480
gggaccctgt gacgaaatcg gcttagcaagg ctgtgctgtc tgccctgttg atggcaggct	6540
tggccctgca gccaggcaact gcccgtgtgt gctactctcg caaageccag gtgagcaacg	6600
aggactgcct gcagggggag aactgcaccc agctgggggaa gcagtgcctgg accgegcgca	6660
tccgcgcagt tggccctctg accgtcatca gcaaaggctg cagcttgaac tgcgtggatg	6720
actcacagga ctactacgtg ggcaagaaga acatcaegtg ctgtgacacc gacttgtgca	6780
acgccagcg ggcccatgcc ctgcagccgg ctgccgcct ccttgcgcgtg ctccctgcac	6840
tccgcgtgt gctctggggc cccggccagc tatagagatc tggggccctaa caaaacaaaa	6900
agatggggttt attccctaaa cttcatgggt tacgttaattt gaaatggggg gacattgcca	6960
caagatcata ttgtacaaaaa gatcaaacac tgttttagaa aacttcctgt aaacaggcct	7020
attgatttggaa aagtatgtca aaggattgtg ggtctttgg gctttgcgc tccatttaca	7080
caatgtggat atccctgcctt aatgcctttg tatgcatgttatacaagctaa acaggcttc	7140
actttctcgc caacttacaa ggcctttcta agtaaacatg acatgaacct ttaccccggt	7200
gctcgccaaac ggcctggctct gtgcctgtt gttgctgacg caaccccccac tggctggggc	7260
ttggccatag gccatcagcg catgcgtggaa acctttgtgg ctccctgcctt gatccataact	7320
gcggaaactcc tagccgcttg ttttgcgc agccggcttg gagcaaaagct cataggaact	7380
gacaattctg tcgtcccttc gcgaaatata acatcggtt gatctacgtt tgatctttt	7440
ccctctgcca aaaattatgg ggacatcatg aagcccccttgg agcatctgac ttctggctaa	7500
taaaggaaat ttatccat tgcaatagtg tgtttggatttttgcgtct ctcactcgga	7560
agc	7563

<210> SEQ ID NO 27

<211> LENGTH: 6396

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 27

ggcgtaatgc tctgccatgt ttacaaccaa ttaaccaattt ctgatttagaa aaactcatcg	60
agcatcaaat gaaaactgcaaa ttatttcata tcaggattat caataccata tttttggaaaa	120
agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgttcc gactcgatca acatcaatac aacattttaa tttccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaaagct tatgcatttc ttccagact tgttcaacag gccagccatt acgctcgatca	360
tcaaaaatcac tcgcataac caaaccgtta ttcatcgatg attgcgcctg agcgagacga	420
aatacgcgtatcgatcaaa aggacaatata caaacaggaa tcaaatgcaaa ccggccgagg	480
aacactgcca ggcgcataac aatatttca cctgaatcag gatatttttc taataccctgg	540
aatgcgtttt tcccgccatcg cgcagttgtt gatcaaccatg catcatcagg agtacggata	600
aaatgcgttga tggtcggaaag aggatcaat tccgtcagcc agtttagtct gaccatctca	660
tctgtacat cattggcaac gctacccttg ccatgtttca gaaacaactc tggcgatcg	720
ggcttcacat acaatcgata gattgtcgca cctgattgccc cgacattatc gcgagccat	780
ttataccat ataaatcagc atccatgtt gaaatataatc gggccctcgaa gcaagacgtt	840
tcccggttggaa tatggctcat aacaccctt gtattactgt ttatgtaaagc agacaggctcg	900

-continued

acaatattgg ctattggcca ttgcatacgt tgtatctata tcataaatatg tacattata	960
ttggctcatg tccaaatatga ccgcctatgtt gacattgatt attgactagt tattaatagt	1020
aatcaattac ggggtcatta gttcatagcc catatatggg gttccgcgtt acataaactta	1080
cggtaaatgg cccgcctggc tgaccgccc acgacccccc cccattgacg tcaataatga	1140
cgtatgttcc catagtaacg ccaataggga cttccattg acgtcaatgg gtggagtatt	1200
tacggtaaac tgcccacttg gcagtagatc aagtgtatca tatgccaatgt ccgcccccta	1260
ttgacgtcaa tgacggtaaa tggccgcct ggcattatgc ccagtagatg accttacggg	1320
actttcttac ttggcagttac atctacgtat tagtcatgc tattaccatg gtgtatgcgt	1380
tttggcagta caccaatggg cgtggatagc ggtttgactc acggggattt ccaagtctcc	1440
accccatgtg cgtcaatggg agtttggttt ggccacaaaaa tcaacgggac tttccaaaat	1500
gtcgtaataa ccccgccccg ttgacgc当地 tggccgttag gctgtacgg tggaggtct	1560
atataaaggcag agctcgatca gtacccgtc agatcgctg gagacgc当地 ccacgctgtt	1620
ttgacctcca tagaagacac cgggaccgat ccagcctccg cggccgggaa cggtgcattt	1680
gaacgeggat tccccgtgcc aagagtgtact caccgtccgg atctcagccaa gcaggatgt	1740
actctccagg gtgggctgg ctccccctgtt caagactcca gggatttgag ggacgctgt	1800
ggctcttc当地 ttacatgtac cttttgc当地 cctcaaccct gactatcttcc caggtcagga	1860
tc当地 cagatc当地 aggggtctgtt attttcttgc tgggtgc当地 agttcaggaa cagtaaacc	1920
tgctc当地 agtgc当地 attc当地 ctc当地 acatctcgatc aatctccgg aggactgggg accctgtgac	1980
gaacatggct agcgegccc gcccgc当地 gctgtgegct gggccgtgg tgctggccgg	2040
tggctctt当地 ctccctggct ctcccttccgg gtgggttataa aatccctcca atgaagctac	2100
taacattact ccaaagcata atatgaaagc atttttggat gaattgaaag ctgagaacat	2160
caagaagttc ttatataatt ttacacatg accacatttgc cagggacag aacaaaactt	2220
tcagcttgc当地 aagcaaatttcc aatccctgtt gaaagaattt ggcctggatt ctgttgc当地	2280
ggcacattat gatgtcctgt tgc当地 taccacc aaataagact catcccaact acatctcaat	2340
aattaatgaa gatggaaatg agatttcaa cacatcatta ttgaaaccac ctccctccagg	2400
atatgaaat gtttggata ttgttaccacc ttgc当地 gcttcttcc aaggaatgcc	2460
agagggcgat ctatgtatg ttaactatgc acgaactgaa gacttcttta aattgaaacg	2520
ggacatgaaa atcaattgtctt ctggaaaatg tgtaattggc agatatgggaa aagttttag	2580
aggaaaataag gttaaaatg cccagctggc agggggccaaa ggagtc当地 tctactccgaa	2640
ccctgctgac tacttgc当地 ctgggggtgaa gtc当地 tccaa gatgggttggaa attttcttgc	2700
agggtgggtgc当地 cagcgtggaa atatccctaa tctgaatggt gcaggagacc ctctcacacc	2760
aggttacccaa gcaaataatgat atgctttagtgc gctgtggaaat gcaaggccctg ttgttctcc	2820
aagtattccctt gttcatccaa ttggataacta tgatgc当地 aagctccctag aaaaatggg	2880
tggctcagca ccaccagata gcagctggag aggaagtctc aaagtgc当地 acaatgttgg	2940
acctggctt当地 actggaaact ttctacaca aaaatgtcaatg atgc当地 acatctaccaa	3000
tgaagtgc当地 agaatttaca atgtgatagg tactctc当地 ggagcactgg aaccagacag	3060
atatgtc当地 ctggggatgc accgggactc atgggtt当地 ggtggatattg accctc当地	3120
tggagcagct gttgttcatg aaattgttag gagctttggaa acactgaaa aggaagggtg	3180
gagacctaga agaacaattt tgtttgc当地 ctgggatgc当地 gaagaatttgc当地 gtcttcttgc当地	3240

-continued

ttctactgag tggcagagg agaattcaag actccttcaa gagcgtggcg tggcttatat	3300
taatgctgac tcatactatag aaggaaacta cactctgaga gttgatttga caccgctgat	3360
gtacagctt gtagacaacc taacaaaaga gctgaaaagc cctgatgaag gctttgaagg	3420
caaatacttt tatgaaaagtt ggactaaaaa aagtccctcc ccagagttca gtggcatgcc	3480
caggataagc aaattgggat ctggaaatga ttttgagggt ttcttccaac gacttggaat	3540
tgcttcaggc agagcacggc atactaaaaa ttgggaaaca aacaaattca gcggctatcc	3600
actgtatcac agtgtctatg aaacatatga gttgggtgaa aagttttatg atccaatgtt	3660
ttaatatac ctcactgtgg cccaggttcg aggagggatg gtgtttgagc tggccattc	3720
catagtgc cctttgatt gtcgagatta tgctgttagt ttaagaaagt atgctgacaa	3780
aatctacagt atttctatga aacatccaca gggaaatgaa acatacagtg tatcattga	3840
ttcactttt tctgcagtaa agaattttac agaaattgct tccaagttca gtgagagact	3900
ccaggacttt gacaaaagca acccaatagt attaagaatg atgaatgatc aactcatgtt	3960
tctggaaaga gcatttattt atcattttagg gttaccagac aggccctttt ataggcatgt	4020
catctatgc ccaaggcagcc acaacaagta tgcaggggg tcatcccag gaatttatga	4080
tgcctgttt gatattgaaa gcaaagtggc cccttccaag gcctggggag aagtgaagag	4140
acagatttt gttcagcct tcacagtgc ggcagctgca gagacttgc gtgaagtagc	4200
cggatccgaa ggttaggggtt cattattgac ctgtggagat gtcgaagaaa acccaggacc	4260
cgcaagcaag gctgtgctgc ttgcctgtt gatggcaggc ttggccctgc agccaggcac	4320
tgccctgctg tgctactcct gcaaaagcccc ggtgagcaac gaggactgcc tgcaggtgg	4380
gaactgcacc cagctgggg agcagtgctg gaccgcgcgc atccgcgcag ttggccctcct	4440
gaccgtcatac agcaaaaggct gcagcttgcgatgc gactcacagg actactacgt	4500
gggcaagaag aacatcacgt gctgtgacac cgacttgcgc aacgcgcgcg gggccatgc	4560
cctgcagccg gctgcggcca tccttgcgc tgccttgcgc tgcgcgcgc tgcctgtgg	4620
accggccag ctatagagat ctggcccta acaaaacaaa aagatgggg tattccctaa	4680
acttcatggg ttacgttaatt ggaagttggg ggacattgcc acaagatcat attgtacaaa	4740
agatcaaaca ctgttttaga aaacttcctg taaacaggcc tattgattgg aaagtatgtc	4800
aaaggattgt gggcttttg ggcttgcgc ctccatttac acaatgtggc tattccctgc	4860
taatgcctt gtatgcatgt atacaagcta aacaggctt cacttctcg ccaacttaca	4920
aggccttct aagtaaacag tacatgaacc ttatccccgt tgctcgccaa cggccctggc	4980
tgtgccaagt gtttgcgtac gcaaccccca ctggctgggg ctggccata ggccatcagc	5040
geatgcgtgg aaccttgcgt gtcctctgc cgatccatac tgccggactc ctgcgcgtt	5100
gttttgcgtc cagccggctc ggagcaaagc tcataggaac tgacaattct gtcgtccct	5160
cgccggaaata tacatgcgtt cgatctacgt atgatctttt tccctctgcc aaaaattatg	5220
gggacatcat gaagccccctt gagcatctga ctctggcta ataaaggaaa tttatccat	5280
ttgcaatagt gtgttggaaat tttttgtgtc tctcactcg aaggaattct gcattaaatga	5340
atcggccaaac ggcggggag aggccggttt cgtattggc gctctccgc ttccctgc	5400
actgactcgc tgcgtcggt cggtcggtc cggcgagcgg tatcagctca ctcacaggcg	5460
gtataacggt tatccacaga atcaggggat aacgcagggaa agaacatgtg agcaaaaggc	5520
cagcaaaagg ccaggaaccg taaaaaggcc gcgttgcgtt cgttttcca taggctccgc	5580
ccccctgacg agcatcacaa aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga	5640

-continued

```

ctataaagat accaggcggtt tccccctgga agtccctcg tgcgctctcc tgttccgacc 5700
ctgccgctta ccggataacct gtccgccttt ctcccctcgg gaagcgtggc gctttctcat 5760
agetcacgct gtaggttatct cagttcggtg taggtcggtc gctccaagct gggctgtgtg 5820
cacgaacccc ccgttcagcc cgaccgctgc gccttateccg gtaactatcg tcttgagtc 5880
aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga 5940
gcgaggtatg taggegggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact 6000
agaagaacag tatttggtat ctgcgcctcg ctgaagccag ttaccttcgg aaaaagagtt 6060
ggtagcttctt gatccggcaa acaaaccacc gctggtagcg gtggttttt tgtttgcag 6120
cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg 6180
tctgacgctc aytggAACGA aaactcacgt taagggattt tggtcatgag attatcaaaa 6240
aggatcttca octagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata 6300
tatgagtaaa otgggtctga cagttacca tgcttaatca gtgaggcacc tatctcagcg 6360
atctgttotat ttcgttcatc catagttgcc tgactc 6396

```

<210> SEQ ID NO 28

<211> LENGTH: 6405

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 28

```

ggcgtaatgc tctgcgcagtg ttacaaccaa ttaaccaatt ctgattagaa aaactcatcg 60
agcatcaaat gaaactgcaa tttattcata tcaggattat caataccata tttttaaaaa 120
agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc 180
tggtatcggt ctgcgttcc gactcgtcca acatcaatac aacctattaa ttcccttcg 240
tcaaaaataa gtttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat 300
ggcaaaaact tatgcatttc tttccagact tggtaacacg gccageccatt acgctcgta 360
tcaaaaatcac tcgcatcaac caaaccgtta ttcatcggtt attgcgcctg agcgagacga 420
aatacgcgat cgctgttaaa aggacaattt caaacaggaa tcaaatacgaa ccggcggcagg 480
aacactgcca ggcgcataac aatattttca cctgaatcgat gatattcttc taatacctgg 540
aatgcgtttt tcccgggat ccgcgtgggt agtaaccatg catcatcagg agtacggata 600
aaatgcttga tggtcggaaagg aggataat tccgtcagcc agtttagtct gaccatctca 660
tctgttaacat cattggcaac gctacccttg ccatgttca gaaacaactc tggcgcatcg 720
ggcttccat acaatcgata gattgtcgca cctgattgcc cgacattatc gcgagccat 780
ttataccat ataaatcagc atccatgttgc gaatttaatc gcccgcctcg gcaagacgtt 840
tcccgttcaa tatggctcat aacacccctt gtattactgt ttatgtaaac agacaggctcg 900
acaatattgg ctattggcca ttgcatacgat tgcataatcgat tacattata 960
ttggctcatg tccaaatatgc ccgcgcattt gacattgttgc attgactgtt tattatgt 1020
aatcaattac ggggtcatta gttcatagcc catatatggaa gttccgcgtt acataactt 1080
cggttaatgg cccgcctggc tgaccggcca acgacccccc cccattgcacg tcaataatga 1140
cgtatgttcc catagtaacg ccaataggaa cttccatttgc acgtcaatgg gtggagtatt 1200
tacggtaaac tgcccacttg gcagtcacatc aagtgtatca tatgccaagt ccgcccccta 1260

```

-continued

ttgacgtcaa tgacggtaaa tggcccgctt ggcattatgc ccagtagatg accttacggg	1320
actttctac ttggcagtac atctacgtat tagtcatacg tattaccatg gtatgcgggt	1380
tttggcagta caccaatggg cgtggatagc ggtttgactc acggggattt ccaagtctcc	1440
accccattga cgtcaatggg agtttgggg ggcacccaaa tcaacgggac tttccaaaat	1500
gtcgtataaa ccccgccccg ttgacgcaa tggcggttag gcgtgtacgg tgggaggct	1560
atataagcg agctcgatca gtgaaaccgtc agatcgectg gagacgcctt ccacgctgtt	1620
ttgacctcca tagaagacac cgggaccgtt ccagcctccg cggccggaa cggtcattt	1680
gaacggegt tccccgtgcc aagagtgact caccgtccgg atctcagcaa gcaggtatgt	1740
actctccagg gtgggcctgg ctccccagt caagactcca gggattttag ggacgctgtt	1800
ggctcttc ttagatgtac cttttgtttt cctcaaccctt gactatcttc caggtcagga	1860
tcccagagtc aggggtctgt atttcctgc tgggtggctcc agttcaggaa cagtaaaccc	1920
tgctccgaat attgccttc acatctcgatc aatctccggc aggactgggg accctgtgac	1980
gaacatggct agcaaggctg tgctgcttgc cctgtttagtgc cctgcagcc	2040
aggcaactgc ctgctgtgtc actcctgc aaagccagggtt agcaacgagg actgcctgca	2100
ggtggagaac tgcacccagc tgggggagca gtgctggacc gcgcgcaccc ggcagttgg	2160
cctcctgacc gtcatcagca aaggctgcag cttgaactgc gtggatgact cacaggacta	2220
ctacgtgggc aagaagaaca tcacgtgtc tgacaccgtt ttgtcaaccc ccagcggggc	2280
ccatgcctc cagccggctg ccgcacatcc tgcgtgtctt cctgcactcg gcctgctgt	2340
ctggggaccc ggccagctt gatcccagac cctgaactttt gatctgttca aactggcagg	2400
cgatgtggaa agcaacccag gccaaatggc aagcgcgcgc cgcccgccgtt ggctgtgcgc	2460
tggggcgctg gtgctggcggtt ttcgttgcggc ttccctttcg ggtggtttat	2520
aaaatccctcc aatgaagcta ctaacatttttcc tccaaagcat aatatgaaat catttttgg	2580
tgaattgaaa gctgagaaca tcaagaagttt cttatataat ttacacaga taccacattt	2640
agcaggaaca gaacaaaactt ttcagcttgc aaagcaaattt caatccaggat ggaaagaattt	2700
tggcctggat tctgttggc tggcacatta tggatgttgc ttgtccatcc caaataagac	2760
tcatccaaac tacatctcaa taattatgtt agatggaaat gagatttca acacatcatt	2820
atttgaacca ctcctccatg gatatggaaa ttgttccatg attgttccac ctttcgttgc	2880
tttctctctt caaggaatgc cagaggcgtt tctgtgtat gttaactatg cacgaactga	2940
agacttctttt aaatttggaaac gggacatgaa aatcaatttgc tctggggaaa ttgttgc	3000
cagatgtgggg aaagtttca gaggaaataa ggttaaaaatt gcccagctgg cagggggccaa	3060
aggagtcattt ctctactccg accctgttgc ctactttgtt cctgggggttga agtccatcc	3120
agatgggttgg aatcttcttgc gaggtgggtt ccagcgttgc aatatccatc atctgttgc	3180
tgcaggagac cctctcacac caggttaccc agcaaatggaa tatgttttata ggcgttgc	3240
tgcagaggctt gttggcttcc caagtatcc tggatgttgc attggataact atgttgcaca	3300
gaagactcttca gaaaaatgg gttggcttgc accaccatg agcagctgg aaggttgc	3360
caaagtgcctt tacaatgtttt gacctggctt tactggaaac ttgttctacac aaaaagtcaa	3420
gatgcacatc cactctacca atgaagtgc aagaatttttac aatgttgc atgttgc	3480
aggagcgttgc gaaccacaca gatatgttgc tctggggatg caccggactt catgggttgc	3540
tgggttgtt gaccctcaga gtggagcgc tggatgttgc gaaatttgc gggatgttgc	3600
aacactgaaa aaggaagggtt ggagaccttgc aagaacaattt ttgttgcac gctggatgc	3660

-continued

agaagaattt ggtcttcttg gttctactga gtgggcagag gagaattcaa gactccttca	3720
agagcgtggc gtggcttata ttaatgctga ctcatactata gaaggaaact acactctgag	3780
agttgattgt acaccgctga tgtacagctt ggtacacaac ctaacaaaag agctgaaaag	3840
ccctgatgaa ggcttgaag gcaaatctct ttatgaaagt tggactaaaa aaagtccctc	3900
cccagagttc agtggcatgc ccaggataag caaattggga tctggaaatg atttttaggt	3960
gttctccaa cgacttggaa ttgcttcagg cagagcacgg tataactaaaa attggaaac	4020
aaacaaatc agcggctatc cactgtatca cagtgttatc gaaacatatg agttggtgga	4080
aaagtttat gatccaatgt ttaaatatca cctcaactgtg gcccaggttc gaggaggat	4140
ggtgttttag ctggccaatt ccatagtgtc ccctttgtat tgtcgagatt atgctgttagt	4200
ttaaagaag tatgctgaca aaatctacag tatttctatg aaacatccac aggaaatgaa	4260
gacatacagt gtatcattt attcaacttt ttctgcagta aagaattta cagaaattgc	4320
ttccaagttc agtgagagac tccaggactt tgacaaaagc aacccaatag tattaagaat	4380
gatgaatgat caactcatgt ttctggaaag agcatttatt gatccattag gtttaccaga	4440
caggccttt tataggcatg tcatactatgc tccaagcagc cacaacaagt atgcagggg	4500
gtcatttcca ggaattttagt atgctctgtt tgatattgaa agcaaagtgg acccttccaa	4560
ggcctgggaa gaagtgaaga gacagattt tggtgcagcc ttcacagtgc aggcagctgc	4620
agagactttg agtgaagtag cctaaagatc tggccctaa caaaacaaaa agatggggtt	4680
attccctaaa cttcatgggt tacgtatgg gaagttgggg gacattgcca caagatcata	4740
ttgtacaaaa gatcaaacac tggtttagaa aacttcctgt aaacaggcct attgattgga	4800
aagtatgtca aaggattgtg ggtctttgg gctttgtgc tccatttaca caatgtggat	4860
atcctgcctt aatgcctttg tatgcatgtc tacaagctaa acaggcttc actttctcgc	4920
caacttacaa ggccttcta agtaaacagt acatgaacct ttacccctt gctcgcaac	4980
ggcctggct gtcggaaagt tttgctgacg caacccccac tggctgggc ttggccatag	5040
gccccatcgcg catgegtgga acctttgtgg ctccctgc gatccatact gccggactcc	5100
tagccgctt tttgctgcg agccggctcg gagcaaagct cataggaact gacaattctg	5160
tctgtcttc gcggaaatat acatcggtt gatctaegta tgatctttt ccctctgcca	5220
aaaattatgg ggacatcatg aagcccttg agcatctgac ttctggctaa taaaggaaat	5280
ttatcttcat tgcaatagtg tggatatttttctcactcgga aggaattctg	5340
cattaaatgaa tcggccaacg cgccgggaga ggcgggttgc gtattggcg ctcttcgct	5400
tccctcgctca ctgactcgct gcgctcggtc gttcggtgc ggcgagcggt atcagctcac	5460
tcaaaggccg taatacggtt atccacagaa tcaggggata acgcaggaaa gaacatgtga	5520
gcaaaaggcc agcaaaaggc caggaaccgt aaaaaggccg cgttgtgc gttttccat	5580
aggctccgcc cccctgacga gcatcacaaa aatcgacgct caagtcagag gtggcgaac	5640
ccgacaggac tataaagata ccaggcggtt cccctggaa gtcctctgt gctctctcct	5700
gttcccgacc tggccgttac cggataccgt tccgccttc tcccttggg aagcgtggcg	5760
ctttctcata gtcacgctg taggtatctc agttcggtgt aggtcggtcg ctccaagctg	5820
ggctgtgtgc acgaaccccc cgttcagccc gaccgctgc cttatccgg taactatcgt	5880
cttgagtcca acccggttaag acacgactta tggccactgg cagcagccac tggtaacagg	5940
attagcagag cgaggtatgt aggcgggtgtc acagagttct tgaagtgggt gcctaactac	6000

US 9,468,672 B2

255**256**

-continued

ggctacacta gaagaacagt atttggtatac tgcgctctgc tgaagccagt taccttcgga	6060
aaaagagttg gtagcttgc atccggcaaa caaacccaccc ctggtagccg tggtttttt	6120
gtttcaagc agcagattac gcgcagaaaa aaaggatctc aagaagatcc tttgatctt	6180
tctacgggt ctgacgctca gtggAACAA aactcacgtt aagggatttt ggtcatgaga	6240
ttatcaaaaa ggatcttac ctagatcctt ttaaattaaa aatgaagttt taaatcaatc	6300
taaaatatat atgagtaaac ttggctgtac agttaccaat gcttaatcag tgaggcacct	6360
atctcagcga tctgtctatt tcgttaccc atagttgcct gactc	6405

<210> SEQ ID NO 29

<211> LENGTH: 6750

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 29

ggcgtaatgc tctgccagt ttacaaccaa ttaaccaatt ctgatttagaa aaactcatcg	60
agcatcaaat gaaactgcaa tttattcata tcaggattat caataccata tttttgaaaa	120
agccgtttct gtaatgaagg agaaaaactca ccgaggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgattcc gactcgtcca acatcaatac aacctattaa tttccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaaact tatgcatttc ttccagact tgttcaacag gccagccatt acgctcgtca	360
tcaaaatcac tcgcatcaac caaaccgta ttcattcgtg attgcgcctg agcggacacga	420
aatacgcgtt cgctgttaaa aggacaatta caaacaggaa tcaaattgcaa ccggcgcagg	480
aacactgcca ggcgcataac aatattttca cctgaatcag gatattctc taatacctgg	540
aatgctgttt tcccggggat cgcaatggtg agtaaccatg catcatcagg agtacggata	600
aaatgcttga tggtcggaaag aggccataat tccgtcagcc agtttagtct gaccatctca	660
tctgtacat cattggcaac gctaccttg ccatgttca gaaacaactc tggcgcatcg	720
ggcttcccat acaatcgata gattgtcgca cctgattgcc cgacattatc gcgagccat	780
ttatacccat ataaatcagc atccatgttg gaatttaatc gggcctcga gcaagacgtt	840
tcccgttgaat tatggctcat aacacccctt gtattactgt ttatgtaaagc agacaggctcg	900
acaatattgg ctattggcca ttgcatacgt tgtatctata tcataatatg tacattata	960
ttggctcatg tccaaatatga ccgcctatgtt gacattgatt attgactagt tattaatagt	1020
aatcaattac ggggtcatta gttcatagcc catatatggc gttccgcgtt acataactta	1080
cggtaaatgg cccgcgttgc tgaccggcca acgacccccc cccattgacg tcaataatga	1140
cgtatgttcc catagtaacg ccaataggga ctttccattt acgtcaatgg gtggagtatt	1200
tacggtaaac tgcccaacttgc gcaatgtacatc aagtgtatca tatgccaatg ccgcggccat	1260
ttgacgtcaa tgacgttaaa tggccgcctt ggcattatgc ccagtgatcg accttacggg	1320
actttccatc ttggcgtacatc atctacgtat tagtcatcgc tattaccatg gtgtgcgg	1380
tttggcgtacatc caccaatggg cgtggatgc gggttgcactc acggggattt ccaagtctcc	1440
accccatgacatc cgtcaatggg agtttggttt ggcacccaaa tcaacggac tttccaaaat	1500
gtcgtaataa ccccgccccg ttgacgcataa tggccgcgtt ggtgtacgg tggggaggct	1560
atataaaggcag agctcgatcgttgc gtaaccgtc agatcgctg gagacggccat ccacgctgtt	1620
ttgacctcca tagaagacac cgggaccgtat ccagcctccg cggccggaa cgggtgcatttgc	1680

-continued

gaacgcggat tccccgtgcc aagagtgact caccgtccgg atctcagcaa gcaggtatgt	1740
actctccagg gtgggcctgg cttecccagt caagactcca gggatttag ggacgctgtg	1800
ggcttcttc ttacatgtac ctttgcttg cctcaacct gactatctc caggtcagga	1860
tcccagagtc aggggtctgt atttcctgc tggtggctcc agttcaggaa cagtaaaccc	1920
tgctccgaat attgectctc acatctcgac aatctcccg aggactgggg accctgtgac	1980
gaacatggct agcattgtgg gaggctggga gtgcgagaag cattccaaac cctggcaggt	2040
gttgtggcc tctcggtgca gggcagtcgtg cggcgggtgt ctggtgacc cccagtgggt	2100
cctcacagct gcccactgca tcaggaacaa aagcgtgatc ttgctgggtc ggcacagctt	2160
gttcatctt gaagacacag gccaggtatt tcaggtcagc cacagttcc cacacccgct	2220
ctacgatatg agcctctgaa agaatcgatt cctcagggca ggtgtatgact ccagecacga	2280
cctcatgctg ctccgcctgt cagagcctgc cgagctcacg gatgctgtga aggtcatgga	2340
cctgcccacc caggagccag cactggggac cacctgtac gctcaggct gggcagccat	2400
tgaaccagag gagttcttga ccccaaagaa acttcagtgt gtggacotcc atgttatttc	2460
caatgacgtg tgtgegcaag ttcaccctca gaaggtgacc aagttcatgc tigtgtgtgg	2520
acgctggaca gggggcaaaa gcacactgctc ggggtgattct gggggccac ttgtctgtaa	2580
tggtgtgtt caaggtatca cgtcatgggg cagtgaacc tggcctgc ccgaaaggcc	2640
ttccctgtac accaagggtgg tgcattaccg gaagtggatc aaggacacca tcgtggccaa	2700
ccccggatcc cagaccctga actttgatct gctgaaactg gcagggatg tggaaagccaa	2760
cccaggccca atggeaagcg cgccgcgcgc gggctggctg tgcgctgggg cgctgggtct	2820
ggcgggtggc ttctttctcc tggcttctt cttcgggtgg tttataaaat cctccatgaa	2880
agctactaac attactccaa agcataatat gaaagcattt ttggatgaat taaaagctga	2940
gaacatcaag aagtttttat ataattttac acagataccca catttagcag gaacagaaca	3000
aaactttcag cttgcaaaagc aaattcaatc ccagtggaaa gaatttggcc tggattctgt	3060
tgagctggca cattatgatg tccctgttgc ctacccaaat aagactcatc ccaactacat	3120
ctcaataatt aatgaagatg gaaatgagat ttcaacaca tcattatttg aaccacccctcc	3180
tccaggatata gaaaatgttt cggatattgt accacccccc agtgccttct ctcctcaagg	3240
aatgccagag ggcgatctag tgtatgttaa ctatgcacga actgaagact tctttaaatt	3300
ggaacgggac atgaaaatca attgctctgg gaaaattgtt attgccagat atggaaagt	3360
tttcagagga aataagggtta aaaatgccc gctggcaggg gccaaaggag tcattctcta	3420
ctccgaccct gctgactact ttgctcctgg ggtgaagtcc tatccagatg gttggaatct	3480
tccctggggat ggtgtccagc gtggaaatata cctaaatctg aatgggtcag gagaccctct	3540
cacaccaggat tacccagcaa atgaatatgc ttataggcgt ggaattgcag aggctgtgg	3600
tcttccaagt attcctgttc atccaattgg atactatgt gcacagaagc tcctagaaaa	3660
aatgggtggc tcagcaccac cagatagcag ctggagagga agtctcaaag tgccctacaa	3720
tgttggacct ggcttactg gaaacttttc tacacaaaaaa gtcaagatgc acatccactc	3780
taccaatgaa gtgacaagaa ttacaatgt gataggtact ctcagaggag cagtggaaacc	3840
agacagatata gtcattctgg gaggtcaccg ggactcatgg gtgtttgggt gtattgaccc	3900
tcagagtggaa gcagctgttg ttcatgaaat tggaggagc ttggaacac tggaaaagga	3960
agggtggaga octagaagaa caattttgtt tgcaagctgg gatgcagaag aatttggct	4020

US 9,468,672 B2

259**260**

-continued

tcttggttct actgagtgaaa cagaggagaa ttcaagactc cttcaagagc gtggcggtggc	4080
ttatattaat gctgactcat ctatagaagg aaactacact ctgagagttt attgtacacc	4140
gctgatgtac agcttggtaac acaacctaac aaaagagctg aaaageccctg atgaaggctt	4200
tgaaggcaaa tctctttatg aaagtggac taaaaaaagt cttccccag agttcagtgg	4260
catgccagg ataagcaaattt tggttatctgg aaatgatTTT gaggtgtttt tcacaacgact	4320
tggaatttgtc tcaggcagag cacggataac taaaaattgg gaaacaaaca aattcagcgg	4380
ctatccactg tatcacagtg tctatgaaac atatgatTTT gtggaaaagt tttatgatcc	4440
aatgtttaaa tatcacctca ctgtggccca gggtcgaggaa gggatgggtt ttgagctggc	4500
caattccata gtgctccctt ttgattgtcg agattatgct gtagtttaa gaaagtatgc	4560
tgacaaaatc tacagtatTTT ctatgaaaca tccacaggaa atgaagacat acagtgtatc	4620
atTTTgattca ctttttctg cagtaaagaa ttttacagaa attgcttcca agttcagtga	4680
gagactccag gactttgaca aaagcaaccc aatagtatTTA agaatgtatga atgatcaact	4740
catgtttctg gaaagagcat ttattgtatcc attagggtta ccagacagc cttttatag	4800
gcatgtcatc tatgtccaa gcagccacaa caagtatgcg ggggagtcat tcccaggaaat	4860
ttatgtatgc ctgtttgata ttgaaagcaa agtggaccct tccaaggcct ggggagaagt	4920
gaagagacag atttatgtt cagccttcac agtgcaggca gctgcagaga ctttgagtga	4980
agtagcctaa agatctgggc cctaaacaaaa caaaaagatg gggttattcc cttaaaatc	5040
tgggttacgt aattggaaatg tgggggacat tgccacaaga tcatattgtt caaaagatca	5100
aacactgttt tagaaaactt cctgtaaaca ggcctattga ttggaaatgtt tgtcaaagga	5160
ttgtgggtct tttgggttt gctgtccat ttacacaatgtt tggatatcct gccttaatgc	5220
cTTTgtatgc atgtatacaa gctaaacagg ctttcaactt ctcgccaact tacaaggcct	5280
ttctaaatgtaa acagtagatc aacctttacc ccgttgcgtcg gcaacggcct ggtctgtgcc	5340
aagtgtttgc tgacgcaacc cccactggct ggggcttggc cataggccat cagcgcatgc	5400
gtggaaacctt tgggttccct ctggcgatcc atactgcggaa actcctagcc gcttgggtt	5460
ctcgcagccg gtctggagca aagctcatag gaactgacaa ttctgtcgcc ctctcgccga	5520
aatatacatac gtttgcgtatc acgtatgtatc tttttccctc tgccaaaaat tatggggaca	5580
tcatgaagcc ctttgagcat ctgacttctg gctaataaaag gaaattttt ttcattgcaaa	5640
tagtgtgtt gatatTTT tggatctc tggaaaggaa ttctgcattt atgaatcgcc	5700
caacgcgcgg ggagaggcgg tttgcgtatt gggcgcttcc cccgttccctc gctcaactgac	5760
tgcgtgcgtt cgggtcgatcg gctgcggcga gcggtatcag ctcactcaaa ggcggtaata	5820
cggttatcca cagaatcagg ggataacgcg gggaaagaaatc tggagccaa aggccagcaa	5880
aaggccagga accgtaaaaaa ggccgcgtt cttggcggtt tccataggct ccgcggccct	5940
gacgagacatc acaaaaatcg acgctcaagt cagagggtggc gaaacccgac aggactataa	6000
agataccagg cgTTTccccc tggaaagctcc ctgcgtgcgtt ctccgtttcc gaccctggcc	6060
cttaccggat acctgtccgc ctttccctt tggggaaagcg tggcgcttcc tcatagctca	6120
cgctgttaggt atctcagttc ggtgttaggtc gttcgctcca agctgggcgtg tggcagcggaa	6180
ccccccgttc agcccgaccg ctgcgcctta tccggtaact atcgcttga gtccaaacccg	6240
gtaaagacacg acttatcgcc actggcagca gccactggta acaggattag cagagcgagg	6300
tatgttaggcg gtgctacaga gttcttgaag tgggtggctta actacggcta cactagaaga	6360
acagtatTTG gtatctgcgc tctgtgtttt ccagttaccc tggaaaaag agttggtagc	6420

-continued

tcttgcgtccg gcaaacaac caccgctgggt agcggtgggtt tttttgttttcaagcagcag	6480
attacgcgcga gaaaaaaagg atctcaagaa gatcctttaa tcttttctac ggggtctgac	6540
gctcagtggaa acgaaaactc acgttaaggg attttggtca tgagattatc aaaaaggatc	6600
ttcacctaga tccttttaaa ttaaaaatga agttttaaat caatctaaag tatatatgag	6660
taaacttggt ctgacagttt ccaatgctta atcagtgagg cacctatctc agcgatctgt	6720
ctatccgtt catccatagt tgccgtactc	6750

<210> SEQ ID NO 30
<211> LENGTH: 6908
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 30	
ggcgtaatgc tctgccgtt ttacaaccaa ttaaccaatt ctgatttagaa aaactcatcg	60
agcatcaaattt gaaactgcaa tttattcata tcaggattat caataccata tttttgaaaa	120
agccgtttct gtatgaagg agaaaactca ccggggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgttcc gactcgatca acatcaatac aacctattaa tttcccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaagct tatgcatttc ttccagact tggtaacag gccagccatt acgctcgatca	360
tcaaaatcac tcgcatcaac caaaccgtta ttcatcgat attgcgcctg agcgagacga	420
aatacgcgtttaa aggacaatta caaacaggaa tcaaatgcaa ccggccggagg	480
aacactgcca ggcgttccat aatattttca cctgaatca gatattcttca taatacctgg	540
aatgctgttt tccccggat cgcgtgggtt agtaaccatg catcatcagg agtaeggata	600
aaatgcttgcgttga tggteggaag aggccataat tccgtcagcc agtttagtctt gaccatctca	660
tctgtacat cattggcaac gctaccccttccatgttca gaaacaactc tggcgatcg	720
ggctcccat acaatcgata gattgtcgca cctgattgc cgacattatc gcgagccat	780
ttataccat ataaatcagc atccatgttgc gaatttataatc gcccgcctcgatcaagacgtt	840
tcccgttgcgttcat aacaccctttt gtttactgtt ttagtgcgttgc agacaggctcg	900
acaatattgg ctattggcca ttgcatacgat tggatctata tcataatatg tacatttata	960
ttggctcatg tccaaatatgc cccgcgttgcgttcat gacattgttatttactgtt tattaaatgt	1020
aatcaattac ggggtcatttta gttcatagcc catatatggat gttccgcgtt acataactta	1080
cggtaatgg cccgcgttgcgttcat gacattgttgcgttcat gacattgttatttactgtt tattaaatgt	1140
cgtatgttcc catatgttgcgttcat gacattgttgcgttcat gacattgttatttactgtt tattaaatgt	1200
tacggtaaac tgcccaatgttgcgttcat gacattgttgcgttcat gacattgttatttactgtt tattaaatgt	1260
ttgacgttcaatggat gggccgttgcgttcat gacattgttatttactgtt tattaaatgt	1320
acttttccat tggcgttcat gacattgttatttactgtt tattaaatgt	1380
tggcgttcat gacattgttatttactgtt tattaaatgt	1440
accccatgttcat gacattgttatttactgtt tattaaatgt	1500
gtcgttcat gacattgttatttactgtt tattaaatgt	1560
atataaaggatcgat gacatgttcat gacattgttatttactgtt tattaaatgt	1620
ttgacgttcaatggat gggccgttgcgttcat gacattgttatttactgtt tattaaatgt	1680

-continued

gaacgcggat tccccgtgcc aagagtgact caccgtccgg atctcagcaa gcaggatgt	1740
actctccagg gtgggcctgg cttecccagt caagactcca gggattttag ggacgcgttg	1800
ggcttcttc ttacatgtac ctttgcctg cctcaaccct gactatctc caggtcagga	1860
tcccagatc aggggtctgt attttctgc tgggttgtcc agttcaggaa cagtaaaccc	1920
tgcctccaat attgcctctc acatctcgac aatctcccg aggactgggg accctgtgac	1980
gaacatggct agcgegcgccc gcccgcgtg gctgtgegtt gggcgctgg tgctggcg	2040
tggcttcttt ctccctcggtc tcctcttcgg gtgggttata aaatcctcca atgaagctac	2100
taacattact ccaaagcata atatgaaagc attttggat gaattgaaag ctgagaacat	2160
caagaagttc ttatataatt ttacacagat accacatttgc cagggAACAG aacaaaactt	2220
tcaagcttgc aagcaatttgc aatcccagt gaaagaattt ggcctggatt ctgttgcgt	2280
ggcacattat gatgtcctgt tgccttaccc aaataagact catcccaact acatctcaat	2340
aattaatgaa gatggaaatg agatttcaaa cacatcatta ttgaaccac ctccctcagg	2400
atatgaaaat gtttccggata ttgttaccacc tttcagtgc ttctctcc aaggaatgcc	2460
agagggcgat ctatgttatg ttaactatgc acgaaactgaa gacttcttta aattggAACG	2520
ggacatgaaa atcaatttgc ctggaaaaat tgtaattgcc agatatgggaa aagtttgc	2580
aggaaataag gttaaaaatg cccagctggc aggggc当地 aggactcatc tctactccga	2640
ccctgctgac tacttgcctc ctgggggtgaa gtcctatcca gatgggttga atcttctgg	2700
aggtgggtgc cagcgtggaa atatcctaaa tctgaatggt gcaggagacc ctctcacacc	2760
aggttaccca gcaaatgaat atgcttatag gctgttgcattt gcaaggagctg ttggcttcc	2820
aagtatttgc gttcatccaa ttggatacta tgatgcacag aagcttgc aaaaaatggg	2880
tggctcagca ccaccagata gcagctggag aggaagtctc aaagtgcctt acaatgttgg	2940
acctggctt actggaaact tttctacaca aaaagtcaag atgcacatcc actctaccaa	3000
tgaagtgaca agaatttaca atgtgatagg tactctcaga ggagcagtgg aaccagacag	3060
atatgtcatt ctggggaggc accgggactc atgggtgttt ggtggatattt accctcagag	3120
tggagcagct gttgttcatg aaattgttagt gagctttggaa acactgaaaa aggaagggtg	3180
gagacctaga agaacaattt tggttgcag ctgggatgc gaagaatttgc ttcttctgg	3240
ttctactgag tggcagagg agaattcaag actccttcaag gacgtggcg tggcttat	3300
taatgtcagtc tcatctatag aaggaaacta cactctgaga gttgatttgc caccgtgtat	3360
gtacagcttgc gtacacaacc taacaaaaga gctgaaaagc cctgtatgc gctttggagg	3420
caaatcttctt tatgaaagtt ggactaaaaaa aagtccctcc ccagagttca gtggcatgcc	3480
caggataaggc aaatttggat ctggaaatga ttttgagggtt ttcttccaaac gacttggaaat	3540
tgcttcaggc agagcacggc atactaaaaa ttgggaaaca aacaaatttca gcccgtatcc	3600
actgttatcac agtgcgttatg aaacatatgc gttgggtggaa aagttttatg atccaatgtt	3660
taaatatcac ctcactgtgg cccaggttcg aggaggatg gtgtttgagc tggccattc	3720
catagtgctc cttttgtt gtcgagatata tgctgttagt ttaagaaatg atgcgtacaa	3780
aatctacagt atttctatga aacatccaca gggaaatgaa acatacagtg tatcatttgc	3840
ttcacttttt tctgcagtaa agaattttac agaaatttgc tccaaatgc gtggagact	3900
ccaggactttt gacaaaagca accaaatgtt attaagaatg atgaatgtc aactcatgtt	3960
tctggaaaga gcatttatttgc atccattagg gttaccagac aggccctttt ataggcatgt	4020
catctatgtc ccaagcagcc acaacaagta tgcaggggag tcatccccag gaatttgc	4080

-continued

tgctctgttt gatattgaaa gcaaagtggc cccttccaag gcctggggag aagtgaagag 4140
 acagatttat gttgcagcct tcacagtgcgca ggcaagctgcgca gagacttgcgta gtaaggtagc 4200
 ctaaagatct gacccctaa cgttactggc cgaagccgcg tggataaagg ccggtgtgcg 4260
 ttgtctata tggatatttc caccatatttgcg cgtcttttgcg acaatgtgag ggcccgaaaa 4320
 cctggccctg tcttcttgac gaggattcctt aggggtctttt cccctctcgc caaaggaatg 4380
 caaggctgtg tgaatgtcgta gaggaaagca gttcctctgg aagcttcttg aagacaaca 4440
 aegtctgttag cggccatggc aaccccccac ctggcgacag gtgcctctgc 4500
 ggccaaaagc cacgtgtata agatacacct gcaaaggcgcc cacaacccca gtgccacggt 4560
 gtgagttgga tagttgtgga aagagtcaaa tggctcteect caagcgtatt caacaaggg 4620
 ctgaaggatg occagaaggt accccattgt atgggatctgc atctggggcc tcgggtgcaca 4680
 tgctttacat gtgttagtc gaggttaaaa aacgtctagg ccccccgaac cacggggacg 4740
 tgggttccctt tgaaaaaca cgtatataat atggccagca aggctgtgct gcttgcctg 4800
 ttgatggcag gcttggccct gcagccagggc actgcctgc tggctactc ctgcaagcc 4860
 caggtgagca acgaggactg cctgcaggtg gagaactgcg cccagctggg ggagcagtgc 4920
 tggaccgcgc gcataccgcgc agttggccctc ctgaccgtca tggcaagg ctgcagcttgc 4980
 aactgegtgg atgactcaca ggactactac gtgggcaaga agaacatcac gtgcgtgtac 5040
 accgacttgt gcaacgcccggccat gcccgcgcg cggctgcgc catccttgcg 5100
 ctgctccctg cactggccct gctgctctgg ggacccggc agctataggg atctggggcc 5160
 taacaaaaca aaaagatggg gttattccctt aaacttcatg gttacgtaa ttggaaagttg 5220
 ggggacatttgcacacaatggc atattgtaca aaagatcaaa cactgttttgcgaaacttcc 5280
 tgtaaacagg octattgatt ggaaagtatg tcaaaggatt gtgggtcttt tgggcttgc 5340
 tgctccattt acacaatgtg gatatcctgc cttaatgcctt ttgtatgcatttgcatacaagc 5400
 taaacaggctt tcactttct cggcaactta caaggcctt ctaagtaac agtacatgaa 5460
 cctttacccc gttgtccggc aacggccctgg tctgtgcggaa gtgtttgcgc acgcaccc 5520
 cactggctgg ggcttggcca taggcattca ggcattgcgtt ggaaccccttgcg tggctccct 5580
 gccgatccat actgeggAAC tcctggccgc ttgttttgcgtt cgcagecggtt ctggagcaaa 5640
 gtcatagga actgacaattt ctgtcgctt ctgcggaaa tatacatcgat ttgcgttgc 5700
 gtatgtctt tttccctctg ccaaaaatta tggggacatc atgaagcccc ttgagcatct 5760
 gacttctggc taataaagga aattttttt cattgcaataa gtgtgttggaa attttttgcg 5820
 tctctcaactc ggaaggattt ctgcattaaat gaatcgccca acgcgcgggg agaggcggtt 5880
 tgcgtattgg ggcgttcccttgc gcttccctgc tcactgcactc gctgcgttgc gtcgttccggc 5940
 tgcggcgagc ggtatcgact cactcaaagg cggtaatacg gttatccaca gaatcagggg 6000
 ataacgcagg aaagaacatg tgagcaaaa gcccaggaaac cgtaaaaagg 6060
 cccgcgttgc ggcgttttc cataggctcc gccccctga cgagcatcac aaaaatcgac 6120
 gtcgttgc gtcgttgc gtcgttgc gtcgttgc gtcgttgc gtcgttgc 6180
 gaaatccctt cgtgcgttgc tctgttccga cccgtccgc taccggatc ctgtccgc 6240
 ttctcccttc gggaaagcggtt ggcgttgc tctgttgc tctgttgc tctgttgc 6300
 tggatgtcgat tggatgtcgat tggatgtcgat tggatgtcgat tggatgtcgat 6360
 ggcgttatac cggtaactat cgttgcgttgc tcaacccgggtt aagacacgcac ttatcgccac 6420

-continued

tggcagcgc cactggtaac aggattagca gagcgaggta ttagggcgt gctacagagt	6480
tcttgaagtg gtggctaacc tacggctaca ctagaagaac agtattttgt atctgcgtc	6540
tgctgaagcc agttacccgc ggaaaaagag ttggtagctc ttgateccggc aaacaaacca	6600
ccgctggtag cggtggttt tttgttgcg acgacgat tacgcgcaga aaaaaggat	6660
ctcaagaaga tccttgcgtt tttctacgg ggtctgacgc tcagtgaaac gaaaactcac	6720
gttaaggat ttggcatg agattatcaa aaaggatctt cacctagatc cttttaaatt	6780
aaaaatgaag tttaaatca atctaaagta tatatgagta aacttggtct gacagttacc	6840
aatgcttaat cagtggaggca cctatctcg cgatctgtct atttcgttca tccatagttg	6900
cctgactc	6908

<210> SEQ_ID NO 31
 <211> LENGTH: 6914
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 31	
ggcgtaatgc tctgccagt ttacaaccaa ttaaccaatt ctgattagaa aaactcatcg	60
agcatcaaat gaaactgcaa ttatttcata tcaggattat caataccata tttttgaaaa	120
agccgtttct gtaatgaagg agaaaaactca ccgaggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgattcc gactcgtcca acatcaatac aacctattaa ttccccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaagct tatgcatttc ttccagact tggtaaacag gccagccatt acgctgtca	360
tcaaaaatcac tcgcatcaac caaaccgtta ttcatcggtt attgcgcctg agcggacgaa	420
aatacgcgtt cgtgttaaa aggacaatta caaacaggaa tcaaattgca ccggcgcagg	480
aacactgcca ggcgtcaac aatattttca cctgaatcag gatatttttc taatactgg	540
aatgctgttt tcccgggat cgcagtggtg agtaaccatg catcatcagg agtacggata	600
aaatgctga tggtcggaag aggatcaat tccgtcagcc agtttagtct gaccatctca	660
tctgttaacat cattggcaac gctacccctt ccatgttca gaaacaactc tggcgatcg	720
ggcttccat acaatcgata gattgtcgca cctgattgcc cgacattatc gcgagccat	780
ttataccat ataaatcagc atccatgttg gaatttaatc gcccctcga gcaagacgtt	840
tcccgttcaa tatggctcat aacaccctt gtattactgt ttatgtaaac agacaggtcg	900
acaatattgg ctattggcca ttgcatacgt tggatctata tcataatatg tacattata	960
ttggctcatg tccaatatga ccgcgtatgtt gacattgtt attgactagt tattaatagt	1020
aatcaattac ggggtcatta gttcatagcc catatatggaa gttcccggtt acataactta	1080
cggttaatgg cccgcgttgc tgaccgccta acgacccccc cccattgacg tcaataatga	1140
cgtatgttcc ctagtaacg ccaataggaa ctttccattt acgtcaatgg gtggagtatt	1200
tacggtaaac tgccccatgtt gcgtacatc aagtgtatca tatgccaatgt ccggccctta	1260
ttgacgtcaa tgacggtaaa tggcccgctt ggcattatgc ccagtacatg accttacggg	1320
actttcttac ttggcgttac atctacgtat tagtcatcg tattaccatg gtgtcggtt	1380
tttggcgttac caccatgggg cgtggatgc gggttgactc acggggattt ccaagtctcc	1440
accccatgtt cgtcaatgggg agtttgggg ggcaccaaaa tcaacgggac tttccaaaat	1500
gtcgtaataa ccccgccccg ttgacgcaaa tggcggttag gcgtgtacgg tggaggtct	1560

-continued

atataaggcag agctcggtta gtgaaccgtc agatcgccctg gagacgccat ccacgctgtt 1620
ttgacctcca tagaagacac cgggaccgat ccagcctccg cggccggaa cggtgcattg 1680
gaacgcccgttccca aagagtgact caccgtccgg atctcagcaa gcaggtatgt 1740
actctccagg gtggccctgg ctccccagt caagactcca gggatttgag ggacgctgtg 1800
ggcttcttc ttacatgtac cttttgcttg cctcaacctt gactatctt caggtcagga 1860
tcccaagtc aggggctgtt atttcctgc tggtggctcc agttcaggaa cagtaaaccc 1920
tgctccgaat attgccttc acatctcgta aatctcccg aggactgggg accctgtgac 1980
gaacatggct agcaaggctg tgctgcttgc cctgtttagt gcagggcttg ccctgcagcc 2040
aggcactgcc ctgctgtgt actctcgaa ageccaggtg agcaacgagg actgectgca 2100
ggtgtggagaac tgcacccagc tgggggagca gtgctggacc ggcgcacatcc ggcgcgttgg 2160
cctcctgacc gtcatcagca aaggctgcag cttgaactgc gtggatgact cacaggacta 2220
ctacgtggcc aagaagaaca tcacgtgtc tgacaccgac ttgtgcaacg ccageggggc 2280
ccatgcctg cagccggctg ccgcacatcc tgcgcgtgtc cctgcactcg gcctgctgct 2340
ctggggaccc gccagctat agagatctga cccccctaacg ttactggccg aagccgcttg 2400
gaataaggcc ggtgtgcgtt tgcctatatg ttatttcca ccatattgcc gtctttggc 2460
aatgtgaggg cccggaaacc tggccctgtc ttcttgacga gcattectag gggctttcc 2520
cctctegcca aaggaatgca aggtctgtt aatgtcgtga aggaaggagt tcctctggaa 2580
gcttcctgaa gacaaacaac gtcgttagcg accctttgcg ggcageggaa ccccccacct 2640
ggcgacaggt gcctctgcgg cccaaagcca cgtgtataag atacacctgc aaaggcggca 2700
caaccccaactt gccacgttgc gagttggata gttgtggaaa gagtc当地atg gctctccca 2760
agcgtattca acaagggct gaaggatgcc cagaaggatcc cccattgtat gggatctgat 2820
ctggggcctc ggtgcacatg ctttacatgt gtttagtgcg gttaaaaaaa cgtctaggcc 2880
ccccgaacca cggggacgtg gtttcctt gaaaaacacg atgataatat ggcacaacc 2940
atggcgcgcc ccccgccgtg gctgtgcgtt gggcgctgg tgctggccgg tggcttttt 3000
ctccteggtc tcctttcgg gtggttata aaatccctcca atgaagctac taacattact 3060
ccaaagcata atatgaaagc attttggat gaattgaaag ctgagaacat caagaaggcc 3120
ttatataatt tacacagat accacatttgc gcaaggacag aacaaaactt tcagttgc 3180
aagcaatttc aatcccaactg gaaagaattt ggcctggatt ctgtttagt ggcacattat 3240
gtatgtcctgt tgccttaccc aaataagact catcccaact acatctcaat aattaatgaa 3300
gatggaaatg agatttcaa cacatcatta tttgaaccac ctcctccagg atatgaaaat 3360
gtttcggata ttgttaccacc tttcagtgct ttctctccctc aaggatgcc agaggccgt 3420
ctagtgatgt ttaactatgc acgaaactgaa gacttcttta aattgaaacg ggacatgaaa 3480
atcaatttgcgat cttggaaaat tgtaatttgcg agatatggaa aagttttcag agggaaataag 3540
gttaaaaatg cccagctggc agggccaaa ggagtcatttgc tctactccga ccctgctgac 3600
tactttgcgtc ctggggtgaa gtcctatcca gatggttggaa atcttctgg aggtgggttc 3660
cagcgtggaa atatcctaaa tctgaatgtt gcaggagacc ctctcacacc aggttaccca 3720
gcaaatgaat atgcttatacg gcgttggaaattt gcagaggctg ttggctttcc aagtatttcc 3780
gttcatccaa ttggatacta tgatgcacag aagctccatg aaaaatggg tggctcagca 3840
ccaccagata gcagctggag aggaagtctc aaagtgcctt acaatgttgg acctggctt 3900

-continued

actggaaact tttctacaca aaaagtcaag atgcacatcc actctaccaa tgaagtgaca	3960
agaatttaca atgtgatagg tactctcaga ggagcagtgg aaccagacag atatgtcatt	4020
ctggggaggc accgggactc atgggtgttt ggtggattt accctcagag tggaggcagct	4080
gttggtcatg aaatttgag gagcttggaa acactgaaaa aggaagggtg gagacctaga	4140
agaacaattt tgtttgcag ctgggatgc gaagaatttgc gtcttcttgc ttctactgag	4200
tggcgaggc agaattcaag actccttcaa gagcgtggc tggcttatata taatgctgac	4260
tcatctatag aaggaaacta cactctgaga gttgatttgc caccctgtat gtacagcttgc	4320
gtacacaacc taacaaaaga gctgaaaagc cctgatgcga gctttgaagg caaatcttt	4380
tatgaaagtt ggactaaaaa aagtccctcc ccagagttca gtggcatgcc caggataagc	4440
aaattggat ctggaaatga ttggaggtt ttcttccaaact gacttggaaat tgcttcaggc	4500
agagcacggt atactaaaaa ttggaaaca aacaaattca gcccgtatcc actgtatcac	4560
agtgtctatg aaacatatga gttggtggaa aagttttagt atccaatgtt taaatatcac	4620
ctcaactgtgg cccagggttcg aggagggtatgtt gtgtttgagc tggccaaatccatgtgc	4680
ccttttgcatt gtcgagatta tgctgttagtt ttaagaaaatgt atgctgacaa aatctacagt	4740
atttctatga aacatccaca ggaaatgaag acatacagtg tatcatttgc ttcaacttttgc	4800
tctgcagtaa agaattttac agaaatttgc tccaagttca gtgagagact ccaggacttgc	4860
gacaaaagca acccaatagt attaagaatgc atgaatgttca aactcatgtt tctggaaaga	4920
gcattttatttgc atccatttgc gttaccagac aggccttttgc ataggcatgtt catctatgttgc	4980
ccaaaggcacc acaacaagta tgcaggggag tcattccatgc gaattttatgc tgctctttgc	5040
gatattgaaa gcaaagtggc cccttccaaag gcctggggag aagtgaagag acagattttgc	5100
gttgcagccct tcacagtgc ggcagctgc gagactttgc gtgaagatgc cttaagatct	5160
ggggccctaac aaaacaaaaa gatggggatc ttccctaaac ttcatggggatc acgtaatttgc	5220
aagttgggggg acattgcccac aagatcatat tgcataaaatgc atcaaaacttgc gtttttagaa	5280
acttcctgttgc aacaggccatc ttgattggaa agtattgttgc aggattgtgg gtcttttgc	5340
ctttgcgtgc ccatttacac aatgtggata tcctgccttgc atgcctttgttgc atgcattgttgc	5400
acaagctaaa caggcttca ctttctgc aacttacaag gcctttctaa gtaaacatgttgc	5460
catgaacccctt taccctcggttgc tcggcaacgc gcctggctgtc tgccaaatgttgc tgctgcacgc	5520
aacccccactt ggctggggctt tggccatagg ccatcagcgc atgcgtggaa cctttgtggc	5580
tcctctgcgc atccatactgc cgaaacttgc agccgcgttgc ttgcgtcgca gcccgtctgg	5640
agcaaaagctc ataggaacttgc acaatttgcgttgc cgtccatgttgc cggaaatata catcgatgttgc	5700
atctacgtat gatcttttgc cctctgc aatattatggg gacatcatgttgc agcccttgc	5760
gcattctgtact tctggctaat aaaggaaattt tattttcattt gcaatagtgtt gttggaaattt	5820
tttgtgtcttc tcactcgaa ggaatttgcatttca attaatgttgc cggccaaacgc gcggggagag	5880
gggggttgcgc tattttggcgc tccttcgc tctcgcttgc tgactcgctgc cgctcggtgc	5940
ttcggcgtgc gcgagcggta tcagctacttgc caaaggcggttgc aatacgggttgc tccacagaat	6000
caggggataaa cgcaggaaatgc aacatgttgc gaaaaggcaca gcaaaaaggcc aggaaccgttgc	6060
aaaaggccgc gttgtggcg tttttccatgc ggctccgc cccgttgc gacatgttgc catcacaaaa	6120
atcgacgctc aagtcaagttgc tggcgaaacc cgcacaggacttgc ataaagatc cagggatgttgc	6180
cccttgc aatgttgc ctttgc ctttgc ttccgcacccttgc gccgttacc ggtatcttgc	6240
ccgccttttc cccttcggaa aatgttgc ctttgc ttttgc ttttgc ttttgc ttttgc ttttgc	6300

-continued

gttcggtgta ggtcggtcgc tccaagctgg gctgtgtgca cgaacccccc gttcagcccg 6360
 accgctgccc ttatccggc aactatcgtc ttgagtccaa cccggtaaga cacgacttat 6420
 cgcactggc agcagccact ggtaacagga ttagcagagc gaggtatgta ggcggtgcta 6480
 cagagttctt gaagtggtgg cctactacg gctacactag aagaacagta tttggtatct 6540
 gcgctctgtc gaagccagtt accttcggaa aaagagttgg tagctttga tccggcaaac 6600
 aaaccaccgc tggtagcggt ggttttttg tttgcaagca gcagattacg cgcagaaaaa 6660
 aaggatctca agaagatctt ttgatctttt ctacggggtc tgacgctcag tggAACGAAA 6720
 actcacgtta agggatTTTg gtcatgagat tatcaaaaag gatcttcacc tagatcTTT 6780
 taaattaaaa atgaagtttt aaatcaatct aaagtatata ttagtAAact tggcttgaca 6840
 gttaccaatg ottaatcgtt gggcaccta tctcagegt ctgtctatTT cgttcatcca 6900
 tagttgcctg actc 6914

<210> SEQ ID NO 32

<211> LENGTH: 5411

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 32

ggcgtaatgc tctgccagtg ttacaaccaa ttaaccaatt ctgattagaa aaactcatcg 60
 agcatcaaat gaaaactgaa tttattcata tcaggattat caataccata tttttgaaaa 120
 agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc 180
 tggtatcggt ctgcgttcc gactcggtca acatcaatac aacctattaa tttccccctcg 240
 tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat 300
 ggcaaaaact tatgcatttc tttccagact tggtaacacg gccagccatt acgctcgta 360
 tcaaaaatcac tcgcatcaac caaaccgtt aatccatcg attgcgcctg agcggacgca 420
 aatacgcgtt cgctgttaaa aggacaatta caaacaggaa tcaaatgcaaa ccggcgagg 480
 aacactgcca ggcgtcaac aatattttca cctgaatcag gatattcttc taatacctgg 540
 aatgctgttt tcccgggat cgcagtggtg agtaaccatg catcatcagg agtaeggata 600
 aaatgcttga tggtcggaaagg aggataat tccgtcagcc agtttagtct gaccatctca 660
 tctgttaacat cattggcaac gctaccTTTccatggtaacactc tggcgcatcg 720
 ggctcccat acaatcgata gattgtcgca cctgattgcc cgacattatc gcgagccat 780
 ttataccat ataaatcagc atccatgttga aatattaatc gggcctcgaa gcaagacgtt 840
 tcccgttgaat tatggctcat aacacccctt gtattactgt ttatgtaaac agacaggctg 900
 acaatattgg ctatggcca ttgcatacg tttatctata tcataatatg tacattata 960
 ttggctcatg tccaaatatgaa cccatgtt gacattgatt attgactagt tattaaatgt 1020
 aatcaattac ggggtcatta gttcatagcc catatatggaa gttccgcgtt acataactta 1080
 cggtaaatgg cccgcgttgc tgaccggca acgacccccc cocattgacg tcaataatgaa 1140
 cgtatgttcc catagtaacg ccaataggaa ctttccattt acgtcaatgg gtggagtatt 1200
 tacggtaaac tgcccacttg gcagttatc aagtgtatca tatgccaatgt ccggccctta 1260
 ttgacgtcaa tgacggtaaaa tggcccgctt ggcattatgc ccagttacatg accttacggg 1320
 actttccatc ttggcagtac atctacgtat tagtcatcg tattaccatg gtgtatgcgg 1380

-continued

tttggcagta caccaatggg cgtggatagc ggtttgactc acggggattt ccaagtctcc	1440
accccatgtg cgtcaatggg agtttgggg ggcacccaaa tcaacgggac tttccaaat	1500
gtcgtaataa cccccccccg ttgacgcaa tggggcggtag gctgtacgg tgggaggct	1560
atataagcag agctcgatc gtgaaccgac agatcgctc gagacgccc ccacgctgtt	1620
ttgaccccca tagaagacac cggggaccat ccaggctccg cggccggaa cggtgcatt	1680
gaacggegat tccccgtgcc aagagtgact caccgtccgg atctcagcaa gcaggatgt	1740
actctccagg gtgggcctgg ctccccagt caagactcca gggatttag ggacgctgtg	1800
ggcttcttc ttacatgtac cttttgcttg cctcaaccct gactatctc caggtcagga	1860
tcccagagtc aggggtctgt atttcctgc tgggtggctcc agttcaggaa cagtaaaccc	1920
tgctccgaat attgcctctc acatctcgac aatctccggc aggactgggg accctgtgac	1980
gaacatggct agcaaggctg tgctgcttc cctgttgatg gcaggcttgg ccctgcagcc	2040
aggcactgccc ctgctgtgtc actcctgcaaa agcccagggtg agcaacgagg actgcctgca	2100
ggtggagaac tgcacccagc tggggggagca gtgctggacc gcgcgcatcc ggcgcgttgg	2160
cctcctgacc gtcatcagca aaggctgcag cttgaactgc gtggatgact cacaggacta	2220
ctacgtgggc aagaagaaca tcacgtgtc tgacaccgac ttgtgcacac ccagcggggc	2280
ccatgcctc cagccggctg ccgcctatctc tgctgctgtc cctgcactcg gcctgctgt	2340
ctggggaccc ggccagctat agagatctga cccctaactt ttactggccg aagccgcctt	2400
gaataaggcc ggtgtcggtt tgtctatatg ttatttccca ccatattgcc gtctttggc	2460
aatgtgaggg cccggaaacc tggccctgtc ttcttgacga gcattcctag gggctttcc	2520
cctctcgcca aaggaatgca aggtctgttg aatgtcgtga aggaagcagt tcctctggaa	2580
gottcttggaa gacaacaacat gtctgttagcg accctttgca ggcagcggaa ccccccacct	2640
ggcgacaggt gcctctgcgg cccaaagcca cgtgtataag atacacctgc aaaggcggca	2700
caaccccaagt gccacgttgt gagttggata gttgtggaaa gagtcaaatg gctctcctca	2760
agcgatttca acaaggggct gaaggatgcc cagaaggatc cccattgtat gggatctgtat	2820
ctggggcctc ggtgcacatg cttaatgtgt gtttagtgcg gttaaaaaaa cgtctaggcc	2880
ccccgaacca cggggacgtg gtttccctt gaaaaacacg atgataatat ggcagcatt	2940
gtggggaggct gggagtgcga gaagcatcc caaccctggc aggtgttgtt ggcctctcg	3000
ggcagggcag tctggggcgg tggctgggtg cacccttcaactt gggctctcac agctgcccac	3060
tgcattcaggaa acaaaacgtt gatctgtgtc ggtcggcaca gttgtttca tcctgaagac	3120
acaggccagg tatttcaggat cagccacacg tteccacacc cgtctacga tatgagcctc	3180
ctgaagaatc gattcctcag gccagggtat gactccagcc acgacccatc gctgtccgc	3240
ctgtcagagc ctgcggagct cacggatgtc gtgaagggtca tggacactgcc caccaggag	3300
ccagcactgg ggaccacactg ctacgcctca ggctggggca gcattgaacc agaggagttc	3360
ttgaccccaa agaaaactca gtgtgtggac ctccatgttta tttccatgaa cgtgtgtgc	3420
caagttcacc ctcagaaggt gaccaagttc atgctgtgtc tggacgcgtc gacagggggc	3480
aaaagcacct gctcggtga ttctggggcc ccacttgcgt gtaatgggtt gcttcaaggt	3540
atcacgtcat ggggcagtga accatgtgcc ctgcccggaaa ggcctccct gtacaccaag	3600
gtgggtgcatt accggaaagtg gatcaaggac accatcggtt ccaaccctg aggtctggg	3660
ccctaacaaa acaaaaagat ggggttattc cctaaacttc atgggttacg taattggaaag	3720
ttgggggaca ttgccacaag atcatattgt acaaaaagatc aaacactgtt ttagaaaact	3780

-continued

tcctgtaaac aggcttattt attggaaaat atgtcaaagg attgtgggtc ttttgggctt	3840
tgcgtgttcca ttacacaat gtggataatcc tgccttaatg cttttgtatg catgtataca	3900
agctaaacag gtttactt tctcgccaaac ttacaaggcc tttctaagta aacagtacat	3960
gaacctttac cccgttgctc ggcaacggcc tggtctgtgc caagtgtttt ctgacgcaac	4020
ccccactggc tggggcttgg ccataggcca tcagcgcattt cggtggaccc ttgtggctcc	4080
tctgcgcata catactgcgg aactccttgc cggtttttt gtcgcagcc ggtctggagc	4140
aaagctcata ggaactgaca attctgttgtt ctttcgtggg aaatatacat cgtttgcattt	4200
taclgttatgtt cttttccctt ctgcggaaaa ttatggggac atcatgaagc cccttggca	4260
tctgacttct ggctaataaaa gggaaattttt tttcatttgcgca atagtgtgtt ggaattttt	4320
gtgtctctca ctgcggaaaggaa attctgcattt aatgaatcgcc ccaacgcgcg gggagaggcg	4380
gttttgtat tggggctctt tccgcatttccctt cgctcacttgc ctgcgtgcgc tcggcggttc	4440
ggctgcggcg agcggatca gctcacttgc agggggat aacggttatcc acagaatcc	4500
gggataacgc agggaaagaac atgtgagca aaggccagca aaaggccagg aaccgtaaaa	4560
aggccgcgtt gctggcggtt ttccatagcc tccggccccc tgacgagcat cacaaaaatc	4620
gacgctcaag tcaaggggtt cggaaacccca caggactata aagataaccag gcgtttcccc	4680
ctggaaagctt ctcgtgcgc tcttcgttcc cgaccctgcg ctttaccggta tacctgtccg	4740
cctttctccc ttccggaaage gtggcgctt ctcatagctt acgctgttagg tatctcagtt	4800
cggtgttagt cgttcgttcc aagctgggtt gtgtgcacgg accccccgtt cagccgcacc	4860
gctgcgcctt atccggtaac tategtcttgc agtccaaaccg ggttaagacac gacttacgc	4920
cactggcgcg agccactggt aacaggatttta gcagagcggag gtatgttaggc ggtgtacag	4980
agttcttgaa gtggggctt aactacggctt aactagaag aacagtattt ggtatctgcg	5040
ctctgttgcgaa gccaggatcc ttccggaaaaa gagttggtag ctcttgcattt ggcaaaacaaa	5100
ccaccgctgg tagcgggtgg tttttgtttt gcaaggcgcgca gattacgcgc agaaaaaaag	5160
gatctcaaga agatcttttgc atcttttcta cgggggtctgc cgctcgtgg aacgaaaact	5220
cacgtttaagg gatttggc attagattttaa caaaaaggat cttcaccttgc atccttttaa	5280
ataaaaatg aagttttaaa tcaatctaaa gtatatatgtt gtaaaatgg tctgacagtt	5340
accaatgtttt aatcgttgcg gcacccatctt cagcgatcttgc tctatttgc tcatccatag	5400
ttgcctgact c	5411

<210> SEQ ID NO 33
 <211> LENGTH: 7694
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 33

ggcgtaatgc tctgcgttcc ttacaaccaa ttaaccaattt ctgattttttt aaactcatcg	60
agcatcaaat gaaactgcaat ttatttccata tcaggattttt caataccata tttttggaaaa	120
agccgtttctt gtaatggagg agaaaaactca ccgaggcgttccataggat ggcaagatcc	180
tggatcggtt ctgcgttccatcc gactcgatcc acatcaataac aaccttattaa tttccctcg	240
tcaaaaataaa ggttatcaag tgagaaatca ccatggatgcgactgaatcc cggtgagaat	300
ggcaaaagct tatgcatttc ttccagact tggtaacacggccattt acgctcgatcc	360

US 9,468,672 B2

279**280**

-continued

tcaaaaatcac tcgcatcaac caaaccgtaa ttcattcgtg attgcgcctg agcgagacga	420
aatacgcgat cgctgttaaa aggacaatta caaacaggaa tcaaatacgaa ccggcgccagg	480
aacactgcca gcgcatcaac aatattttca cctgaatcag gatattcttc taatacctgg	540
aatgctgttt tcccgggat cgcagtggtg agtaaccatg catcatcagg agtacggata	600
aatatgctga tggtcggaaag aggataaat tccgtcagcc agtttagtct gaccatctca	660
tctgttaacat cattggcaac gctacccctt ccatgttca gaaacaactc tggcgcatcg	720
ggcttccat acaatcgata gattgtcga cctgattgcc cgacattatc gcgagccat	780
ttataccat ataaatcagc atccatgttgc gaatttaatc gcggcctcga gcaagacgtt	840
tcccgttcaa tatggctcat aacacccctt gtattactgt ttatgtaaagc agacaggctg	900
acaatattgg ctattggcca ttgcatacgt tgtatctata tcataatatg tacattata	960
ttggctcatg tccaaatatga cccatgttgc gacattgattt attgactagt tattatagt	1020
aatcaattac ggggtcatta gttcatagcc catatatggaa gttccgcgtt acataactta	1080
cgttaaatgg cccgcctggc tgacccggca acgacccccc cccattgacg tcaataatga	1140
cgtatgttcc catagtaacg ccaataggga cttccatttgc acgtcaatgg gtggagtt	1200
tacggtaaac tggccacttgc gcagttacatc aagtgtatca tatgccaagt cccccccta	1260
ttgacgtcaa tgacggtaaa tggccgcctt ggcattatgc ccagttacatg accttacggg	1320
actttccatc ttggcgttac atctacgtat tagtcatcgc tattaccatg gtgtgcgggt	1380
tttggcagta caccaatggg cgtggatagc ggtttgcactt acggggattt ccaagtctcc	1440
accccatgta cgtcaatggg agtttgcctt ggcacccaaa tcaacgggac tttccaaaat	1500
gtcgtataaa ccccgccccg ttgacgc当地 tggcggttag gcgtgtacgg tggaggtct	1560
atataaggcag agctcgaaaa ttgaaaccgtc agatcgcttgc gagacgcctt ccacgctgtt	1620
ttgacctcca tagaagacac cgggaccgtt ccagcctccg cggccggaa cgggtcattt	1680
gaacgcggat tccccgtgcc aagagtgttgc caccgtccgg atctcagccaa gcaggatgt	1740
actctccagg gtgggcctgg ctccccctgtt caagactcca gggatttgag ggacgctgt	1800
ggctttcttc ttacatgttgc ctccatgttgc cctcaacccctt gactatcttc caggtcagg	1860
tcccagatgttcc aggggtctgtt attttcttgc tggggctcc agttcaggaa cagtaaaaa	1920
tgcgtccaaat attgcgttcc acatctcgcc aatctcccgcc aggaactgggg accctgtgt	1980
gaacatggctt agcattgtgg gaggctggaa gtgcgagaag cattccaaac cctggcagg	2040
gtttgtggcc tctcgtggca gggcgttgc cggcgggtt ctgggtcacc cccagggtt	2100
cctcacatgtt cccactgca tcaggaacaa aagcgttgc ttgctgggtc ggcacagctt	2160
gtttcatctt gaagacacag gccaggatattt tcaggtcaggc cacatcttc cacacccctt	2220
ctacgtatgtt agcctcttgc agaattcgattt cctcaggccaa ggtgtatgtt ccacgcacgtt	2280
cctcatgtt ctcgcctgtt cagacgttgc cggcgttgc gatgtgttgc aggtcatgtt	2340
cctggccacc caggagccat cactggggac caccgttgc acgttgcaggctt gggcaggat	2400
tgaaccatgtt gaggatgttgc ccccaaaaacttgcgtt gttggacccctt atgttatttgc	2460
caatgacgtt gttgtgttgc ttccatgttgc gaagggttgc aagttcatgtt tttgtgttgc	2520
acgttgcgtt gggggcaaaa gcaatgttgc ggggttgcattt gggggccac ttgtgttgc	2580
ttgggtgttgc ttccatgttgc cgttgcgtt gttgtgttgc cggaaaggcc	2640
ttccatgttgc accaagggttgc tgcattaccgtt gaaatggatc aaggacacca tcgtggccaa	2700
ccccggatcc cagaccctgtt actttgttgc tgcgttgcaggctt gggggccac ttgtgttgc	2760

-continued

cccgaggcca atggcaagcg cgccgcgccc gcgctggctg tgcgctgggg cgctggct	2820
ggcggtggc ttcttctcc tcggcttcct cttcggtgg ttataaaaat cctccatga	2880
agctactaac attactccaa agcataatat gaaagcattt ttggatgaat tgaaagctga	2940
gaacatcaag aagtcttat ataattttac acagataccca catttagcag gaacagaaca	3000
aaacttcag cttgcaaagc aaattcaatc ccagtggaaa gaatttggcc tggattctgt	3060
ttagctggca cattatgatc tcctgttgc ctacccaaat aagactcatc ccaactacat	3120
ctcaataatt aatgaagatg gaaatgagat tttcaacaca tcattatttg aaccaccc	3180
tccaggatata gaaaatgttt cgatattgtt accacccccc agtgctttct ctcctcaagg	3240
aatgccagag ggcgatctag tgtatgttaa ctatgcacga actgaagact tctttaaatt	3300
ggaacgggac atgaaaatca attgctctgg gaaaattgtt attgcccagat atggaaagt	3360
tttcagagga aataaggtaa aaaatgcaca gctggcaggg gccaaaggag tcattctta	3420
cctcgaccct gctgactact ttgctctgg ggtgaagtcc tatccagatg gttggatct	3480
tcctggaggt ggtgtccagc gtggaaatat cctaaatctg aatggtgcag gagaccctct	3540
cacaccaggat taccagccaa atgaatatgc ttataggcgtt ggaattgcag aggctgttgg	3600
tcttccaagt attcctgttc atccaatttg atactatgtt gcacagaagc tcctagaaaa	3660
aatgggtggc tcagcaccac cagatagcag ctggagggag agtctcaag tgccctacaa	3720
tgttggacct ggcttactg gaaacttttc tacacaaaaa gtcaagatgc acatccactc	3780
taccaatgaa gtgacaagaa ttataatgtt gataggtact ctcagaggag cagtggacc	3840
agacagatata gtcattctgg gaggtcaccc ggactcatgg gtgtttgggt gtattgaccc	3900
tcagagtggc gcagctgtt ttcattgtt gttgaggagc ttggaaacac tgaaaaagga	3960
agggtggaga octagaagaa caatttgtt tgcaagctgg gatgcagaag aatttggct	4020
tcttggttct actgagtgcc cagaggagaa ttcaagactc cttcaagagc gtggcggtgg	4080
ttatattaat gctgactcat ctatagaagg aaactacact ctgagatgtt attgtacacc	4140
gctgatgtac agcttggcac acaacctaacc aaaaagactg aaaaagccctg atgaaggctt	4200
tgaaggccaa tctctttatg aaagttggac taaaaaaatgtt cttccccccat agttcagttgg	4260
catgcccagg ataagccaaat tggatctgg aaatgatttt gaggtgttct tccaacgact	4320
tggaaattgtt tcaggcagag cacggatac taaaaattgg gaaacaaaca aattcagccg	4380
ctatccactg tatcacactg tctatgaaac atatgatgtt gtggaaaatgtt tttatgatcc	4440
aatgtttaaa tatcacctca ctgtggccca gggtcgagga gggatgggtt ttgagctggc	4500
caattccata gtgctccctt ttgattgtcg agattatgtt gtatgtttaa gaaagtatgc	4560
tgacaaaatc tacagtatcc ctatgaaaca tccacaggaa atgaagacat acagtgtatc	4620
atttgattca ctttttctg cagtaaagaa ttttacagaa attgcttcca agttcagttga	4680
gagactccag gactttgaca aaagcaaccc aatagtatca agaatgtatgc atgatcaact	4740
catgtttctg gaaagagcat ttattgtatcc attagggtta ccagacaggc cttttatag	4800
gcatgtcatac tatgtccaa gcagccacaa caagtatgc gggatggcat tccaggaaat	4860
ttatgtatgtt ctgtttgata ttgaaagca agtggaccct tccaaaggcct ggggagaagt	4920
gaagagacag atttatgtt cagccctcac agtgcaggca gtcgcagaga ctttgatgt	4980
agtagcctaa agatctgacc ccctaaccgtt actggccgaa gccgcttggaa ataaggccgg	5040
tgtgcgtttg tctatgtt attttccacc atattgcgtt cttttggcaat tggaggcc	5100

-continued

cggaaacctg	gccctgtctt	cttgacgagc	attccttaggg	gtctttcccc	tctcgccaaa	5160
ggaaatgcaga	gtctgtgaa	tgtcgtgaag	gaaggcgttc	ctctggaaac	ttcttgaaga	5220
caaacaacgt	ctgtagcgac	ccttgcagg	cagcggacc	ccccacctgg	cgacaggtgc	5280
ctctgeggcc	aaaagccacg	tgtataagat	acacctgcaa	aggcggcaca	accccagtgc	5340
cacgttgtga	gttggatagt	tgtggaaaga	gtcaaatggc	tctcctcaag	cgtattcaac	5400
aaggggctga	aggatgccc	gaaggatccc	cattgtatgg	gatctgatct	ggggectcg	5460
tgcacatgct	ttacatgtgt	ttagtcgagg	ttaaaaaaacg	tctaggcccc	ccgaaccacg	5520
gggacgttgt	tttccttga	aaaacacat	gataatatgg	ccagcaaggc	tgtgtgtctt	5580
gccctgttga	tggcaggctt	ggccctgcag	ccaggcactg	ccctgtgtg	ctactcctgc	5640
aaagcccaagg	tgagcaacga	ggactgcctg	caggtggaga	actgcaccca	gctggggag	5700
cagtgttgg	ccgcgcgc	ccgcgcagtt	ggcctcttga	ccgtcatcag	caaaggctgc	5760
agcttgaact	gcgtggatga	ctcacaggac	tactacgtgg	gcaagaagaa	catcacgtgc	5820
tgtgacacccg	acttgcgaaa	cgccagcggg	gcccattggcc	tgcagccggc	tgcggccatc	5880
cttgcgtgc	tccctgcact	cggcctgtctg	ctctggggac	ccggccagct	atagggatct	5940
ggggccta	aaaacaaaaaa	gatggggta	ttccctaaac	ttcatgggtt	acgtaattg	6000
aagttgggg	acattgccac	aagatcatat	tgtacaaaag	atcaaacact	gttttagaaa	6060
acttcctgt	aacaggccta	ttgattggaa	agtatgtcaa	aggattgtgg	gtcttttggg	6120
ctttgtct	ccatttacac	aatgtggata	tcctgcctt	atgcctttgt	atgcatgtat	6180
acaagctaa	caggcttca	ctttctcgcc	aacttacaag	gcctttctaa	gtaaacagta	6240
catgaacctt	taccccggtt	ctcggcaacg	gcctggctcg	tgccaaagtgt	ttgctgacgc	6300
accccccaact	ggctggggct	tggccatagg	ccatcagcgc	atgcgtggaa	cctttgtggc	6360
tcctctgcgc	atccatactg	cggaaactcct	agccgcttgc	tttgctcgca	gccggctcg	6420
agcaaaagctc	ataggaactg	acaattctgt	cgtcctctcg	cggaaatata	catcgttcg	6480
atctacgtat	gatcttttc	cctctgc当地	aaattatggg	gacatcatga	agccccctga	6540
gcatctgact	tctggctaat	aaaggaaatt	tattttattt	gcaatagtgt	gttggatatt	6600
tttgggtctc	tcactcgaa	ggaattctgc	attaatgaat	cggccaaacgc	gcggggagag	6660
gggggttgcg	tattggcgcc	tcttcgc当地	cctcgctc	tgactcgctg	cgctcggctcg	6720
ttcggctgcg	gcgagcggt	tcagctca	caaaggcggt	aatacggta	tccacagaat	6780
caggggataa	cgcaggaaag	aacatgttag	caaaggcc	gaaaaggcc	aggaaccgta	6840
aaaaggccgc	gttgetggcg	ttttccata	ggctccgc	ccctgacgag	catcacaaaa	6900
atcgacgctc	aagtca	ggggaaacc	cgacaggact	ataaaagatac	caggcg	6960
cccttggaa	ctccctcg	cgctctctg	ttccgaccct	gcccgttacc	ggataacctgt	7020
ccgccttct	cccttggaa	agcgtggc	tttctcatag	ctcacgtgt	aggtatctca	7080
gttcgggt	gttcgttgc	tccaa	gctgtgtc	cgaa	ccccccgtt	7140
accgctgc	cttatccgt	aactatcg	ttgagtc	cccgta	cacgacttat	7200
cgcactggc	agcagccact	ggtaacagga	ttagcagac	gaggatgt	ggcgggtct	7260
cagagttctt	gaagtgg	cctaactacg	gctacactag	aagaacagta	tttggatct	7320
gegcgtctgt	gaagecagtt	accttcggaa	aaagagtgg	tagcttga	tccggcaac	7380
aaaccaccgc	tggttagcggt	ggttttttt	tttgcaagca	gcagattacg	cgcagaaaa	7440
aaggatctca	agaagatcct	ttgatcttt	ctacgggg	tgacgtc	tggaacgaaa	7500

-continued

actcacgtta	agggattttg	gtcatgagat	tatcaaaaag	gatcttacc	tagatcc	7560
taaaataaaa	atgaagtttt	aaatcaatct	aaagtatata	ttagttaact	tggtctgaca	7620
gttaccaatg	cttaatcagt	gaggcaccta	tctcagcgat	ctgtctat	cgttcatcca	7680
tagttgcctg	actc					7694

<210> SEQ ID NO 34
<211> LENGTH: 7182
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 34												
ggcgtaatgc	tctgccagt	ttacaaccaa	ttaaccaatt	ctgattagaa	aaactcatcg	60						
agcatcaa	at gaaactgcaa	tttattcata	tcaggattat	caataccata	ttttgaaaa	120						
agccgttct	gtaatgaagg	agaaaactca	ccgaggcagt	tccataggat	ggcaagatcc	180						
tggtatcggt	ctgcgattcc	gactcg	tca	acatcaatac	aacctattaa	tttccc	ctcg	240				
tcaaaaataa	ggttatcaag	tgagaaatca	ccatgagt	ga	cgactgaatc	cggtgagaat	300					
ggcaaaagct	tatgcatttc	tttccagact	tgttcaacag	gocagecatt	acgctcg	tca	360					
tcaaaatcac	tcgcatcaac	caaaccgtt	ttcatcg	attgcgc	ctg	agc	420					
aatacgcgat	cgctgttaaa	aggacaatta	caa	acaggaa	tcaa	atgc	aa	ccgg	ggcagg	480		
aacactgcca	gcgc	atcaac	aatat	ttca	cctg	aatc	ttcg	540				
aatgctgtt	tcccg	ggat	cgc	agt	tcg	at	catc	agg	gtac	ggata	600	
aaatgctgtt	tggtcg	gaag	cataa	at	ccgtc	ag	tttagt	ct	gacc	atct	ca	660
tctgt	aacat	catt	ggca	ac	g	ctt	cc	ttc	aa	act	cg	720
ggcttccat	acaatcgata	gatt	gtc	ga	cct	gatt	gc	catt	atc	tcg	780	
ttataccat	ataa	atc	atc	at	tt	ccat	gtc	g	aa	aca	act	840
tcccg	tat	gg	tc	at	cc	cc	tt	gt	tt	at	atc	900
acaatattgg	ctatt	ggc	ca	tt	gc	atacgt	tgt	at	ctata	at	tg	1020
ttggctcat	tcca	at	at	at	ga	ccat	gt	at	at	at	at	1080
aatcaattac	gggg	t	ccat	at	gtt	at	at	gt	at	at	at	1140
cggtaatgg	ccc	cc	ct	gg	cc	ac	cc	at	cc	at	at	1200
cgtatgttcc	cat	at	at	aa	cc	at	gg	at	tt	at	at	1260
tacggtaaac	tgcc	cc	actt	tg	gc	at	ac	at	tt	ca	at	1320
ttgacgtcaa	tgac	gg	ttaa	gg	cc	at	tt	ac	tt	ca	at	1380
acttccat	ttgg	c	at	gt	cc	at	gt	at	cc	at	gt	1440
tttggcagta	cac	ca	at	gg	gg	at	ttt	gg	gg	at	ttt	1500
accccattga	cgt	ca	at	gg	gg	at	ttt	cc	aa	at	ttt	1560
gtcgtaataa	cccc	cc	cc	cc	tt	gg	cc	gg	tt	gg	gg	1620
atataa	ag	ct	cg	tt	ta	gt	aa	cc	tt	cc	at	1680
ttgacctcca	taga	ag	ac	ac	cc	gg	cc	cc	gg	gg	at	1740
gaacgcggat	cccc	cc	gt	cc	gg	cc	gg	at	cc	gg	at	1800
acttccagg	gtgg	cc	ct	gg	cc	at	tt	gg	at	tt	gg	1860

-continued

ggctttctc ttacatgtac ctttgcttgcctcaaccct gactatcttc caggtcagga	1860
tcccagagtc aggggtctgt atttcctgc tggggctcc agttcaggaa cagtaaaccc	1920
tgctccgaat attgectctc acatctcgac aatctccgac aggactgggg accctgtgac	1980
gaacatggct acattgtgg gaggctggga gtgcgagaag cattccaaac cctggcaggt	2040
gtttgtggcc tctcggtggca gggcggctgtc cgccgggtgt ctgggtgacc cccagggtgg	2100
cctcacatgc gcccactgca tcaggaacaa aagcgtgatc ttgctgggtc ggcacagctt	2160
gtttcatcct gaagacacag gccaggtatt tcaggtcagc cacagttcc cacaccgct	2220
ctacgatatg agcctcctga agaatcgatt cctcaggcca ggtgatgact ccagccacga	2280
cctcatgctg ctccgcctgt cagagcctgc cgagctcagc gatgctgtga aggtcatgg	2340
cctggccacc caggagccag cactggggac cacctgtac gcctcaggct gggcagcat	2400
tgaaccagag gagttcttgc cccaaagaa acttcagtgt gtggacctcc atgttatttc	2460
caatgacgtg tgtgcgcaag ttccccctca gaaggtgacc aagttcatgc tgggtgctgg	2520
acgctggaca gggggcaaaa gcacctgctc gggtgattct gggggccac ttgtctgtaa	2580
tgggtgtctt caaggttatca cgtcatgggg cagtgacca tggccctgc ccgaaaggcc	2640
ttccctgtac accaagggtgg tgcattaccg gaagtggatc aaggacacca tggggccaa	2700
ccccggatcc cagaccctga actttgatct gctgaaactg gcaggcgatg tggaaagcaa	2760
cccaggccca atggcaagcg cgccgcgccc gcgcgtggctg tgcgcgtgggg cgctgggtct	2820
ggcgggtggc ttctttctcc tcggcttctt cttcggtgg tttataaaat cctccaaatga	2880
agctactaac attactccaa agcataatat gaaagcattt ttggatgaat tgaaagctga	2940
gaacatcaag aagttttat ataattttac acagatacca cattttagcag gaacagaaca	3000
aaactttcag ttgcggaaacgc aaattcaatc ccagtgaaa gaatttgccc tggattctgt	3060
ttagctggca cattatgtac ttctgttgc ctacccaaat aagactcatc ccaactacat	3120
ctcaataatt aatgaagatg gaaatggat tttcaacaca tcattatttg aaccacccctcc	3180
tccaggatata gaaaatgttt cggatattgt accaccccttc agtgcatttc ctccctcaagg	3240
aatgcccggag ggcgatctag tggatgtttaa ctatgcacga actgaagact tctttaaatt	3300
ggAACGGGAC atgaaaatca attgctctgg gaaaattgtt attgcccggat atggggaaagt	3360
tttcagggaa aataagggtt aaaaatggccca gctggcagggg gccaaaggag tcattctcta	3420
ctccgaccct gctgactact ttgcctctgg ggtgaagtcc tatccagatg gttggatct	3480
tcttgggggtt ggtgtccagc gtggaaatat cctaaatctg aatgggtcag gagaccctct	3540
cacaccagggt tacccagcaa atgaatatgc ttataggcgt ggaattgcag aggctgtgg	3600
tcttccaagt attcctgttc atccaattgg atactatgt gcacagaacgc tccttagaaaa	3660
aatgggtggc tcagcaccac cagatagcag ctggagggaa agtctcaag tgccctacaa	3720
tgttggacct ggctttactg gaaacttttc tacacaaaaaa gtcaagatgc acatccactc	3780
taccaatgaa gtgacaagaa ttacaatgt gataggtact ctcagaggag cagtggaaacc	3840
agacagatgtcattctgg gaggtcaccg ggactcatgg gtgtttgggt gtattgaccc	3900
tcagagtgga gcagctgttg ttcatgaaat tggaggacac tgaaaaagga	3960
agggtggaga octagaagaa caatttgtt tgcaagctgg gatgcagaag aatgggtct	4020
tcttgggtct actgagtggtt cagaggagaa ttcaagactc cttcaagacgc gtggcgtggc	4080
ttatattaat gctgactcat ctatagaagg aaactacact ctgagagttt attgtacacc	4140
gtgtgttac agcttggtaac acaacctaacc aaaaagagctg aaaagccctg atgaaggctt	4200

-continued

tgaaggcaaa tctctttatg aaagttggac taaaaaaaaagt cttccccag agttcagtgg	4260
catgcccagg ataagcaa at tggatctgg aaatgattt gagggttct tcacaacgact	4320
tggaaattgtc tcaggcagag cacggatac taaaaattgg gaaacaaaca aattcagcg	4380
cataccactg tatcacagtg tctatgaaac atatgagttt gtggaaaatg tttatgatcc	4440
aatgtttaaa tatcacctca ctgtggcca gggtcgagga gggatgggt ttgagctggc	4500
caattccata gtgctccctt ttgattgtcg agattatgtc gtagtttaa gaaagtatgc	4560
tgacaaaatc tacagtattt ctatgaaaca tccacaggaa atgaagacat acagtgtatc	4620
atttgattca ctttttctg cagtaagaa ttttacagaa attgcttcca agttcagtga	4680
gagactccag gactttgaca aaagcaaccc aatagtatta agaatgtatga atgatcaact	4740
catgtttctg gaaagagcat ttattgatcc attagggtt ccagacaggc cttttatag	4800
geatgtcatc tatgtccaa gcagccacaa caagtatgca ggggagtcat tcccaggaat	4860
ttatgatgtc ctgtttgata ttgaaagcaa agtggaccct tccaaggcct ggggagaagt	4920
gaagagacag atttatgttg cagcattcac agtgcaggca gtcgcagaga ctttgagtga	4980
agtagecggta tccgaaggta ggggttcatt attgacctgt ggagatgtcg aagaaaaccc	5040
aggaccgcgca agcaaggctg tgctgcttc cctgttgatg gcaaggctgg ccctgcagcc	5100
aggcaactgcc ctgctgtgct actctgtcaa agcccaagggt agcaacgggg actgcctgca	5160
ggtgtggagaac tgcaaccage tgggggagca gtgctggacc ggcgcacatcc ggcgcagtgg	5220
cctccctgacc gtcatcagca aaggctgcag cttgaactgc gtggatgact cacaggacta	5280
ctacgtgggc aagaagaaca tcaacgtgtc tgacacccac ttgtcaacg ccageggggc	5340
ccatgcctcg cagccggctg ccgcacatct tgctgtgtc cctgcactcg gcctgctgct	5400
ctggggaccc ggcctat agagatctgg gccctaacaa aacaaaaaga tgggttatt	5460
ccctaaacct catgggttac gtaattggaa gttggggac attgccacaa gatcatattt	5520
tacaaaagat caaacactgt tttagaaaat ttcctgtaaa caggcctatt gatggaaag	5580
tatgtcaaaat gattgtgggt cttttgggt ttgctgtcc atttacacaa tgtggatatc	5640
ctgccttaat gccttgtat gcatgtatac aagctaaaca ggcttcaact ttctegccaa	5700
cttacaaggc ctttctaaat aaacagtaca tgaaccttta cccccgtgtc cggcaacggc	5760
ctggctgtg ccaagtgttt gctgacgcaa cccccactgg ctggggcttg gccataggcc	5820
atcagegcacat gctgtggacc tttgtggcct ctctgcggat ccatactgcg gaactccat	5880
ccgcttgcgtt tgctcgac cggcttgcggag caaagctcat aggaactgac aattctgtcg	5940
tcctctcgcg gaaatataca tcgtttcgat ctacgtatga tcttttccc tctgcacaaa	6000
attatggggat catcatgaag ccccttgac atctgacttc tggctaaatggaaattttta	6060
ttttcattgc aatagtgtgt ttgaaatttt tggatcttc actctggaaagg aattctgtcat	6120
taatgaatcg gccaacgcgc ggggagagggc ggtttgcgtt ttggggcgtc ttccgcttcc	6180
tcgctcaactg actcgctgcg ctggctcggtt cggctgcggc gagcggatc agctcaactca	6240
aaggcggtaa tacgggttac cacagaatca gggataacg caggaaagaa catgtgagca	6300
aaaggccagc aaaaggccag gaaccgtaaa aaggcccggt tgctggcggtt tttccatagg	6360
ctccgcggccctt ctgacgagca tcacaaaaat cgacgctcaa gtcagagggt ggcggaaacccg	6420
acaggactat aaagatacca ggcgtttccc cctggaaagct ccctcgctgcg ctctccgtt	6480
ccgaccctgc cgcttaccgg atacctgtcc gcctttctcc ctgcggaaag cgtggcgctt	6540

-continued

tctcatagct cacgctgttag gtatctcagt tcgggtgttagg tgcgttcgtc caagctgggc	6600
tgtgtgcacg aaccccccgt tcagccgcac cgctgcgcct tatccggtaa ctatcgctt	6660
gagtccaacc cggtaagaca cgacttatcg ccactggcag cagccactgg taacaggatt	6720
agcagagcga ggtatgttagg cggtgctaca gagttcttga agtggtgccc taactacggc	6780
tacactagaa gaacagtatt tggtatctgc gctctgtga agccagttac ctccggaaaa	6840
agagttggtt gctcttgcac cggcaaaacc accaccgctg gtatcggtgg ttttttgg	6900
tgcaagcgc agattacgcg cagaaaaaaa ggatctcaag aagatctttt gatctttct	6960
acggggctcg acgctcagtgc gaacgaaaac tcacgttaag ggattttgg catgagatta	7020
tcaaaaagga tcttcaccta gatccttttta aattaaaaat gaagttttaa atcaatctaa	7080
agtatatatg agtaaaacttgc tgcgtacatg taccaatgtc taatcgtga ggcacccatc	7140
tcagcgatct gtctatttcg ttcatccata gttgcctgac tc	7182

<210> SEQ_ID NO 35

<211> LENGTH: 7182

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 35

ggcgtaatgc tctgccagt ttacaaccaa ttaaccaatt ctgatttagaa aaactcatcg	60
agcatcaaat gaaactgcaa tttattcata tcaggattat caataccata tttttgaaaa	120
agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgattcc gactcggtca acatcaatac aacctattaa tttccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaagct tatgcatttc tttccagact tggtaaacag gccagccatt acgctcgta	360
tcaaaatcac tcgcatcaac caaaccgtt atcattcgtg attgcgcctg agcggacgca	420
aatacgcgat cgctgttaaa aggacaatta caaacaggaa tcaaatgcaa ccggcgcagg	480
aacactgcca ggcgcataac aatattttca cctgaatcg gatattcttc taataacctgg	540
aatgctgttt tccccggat cgcaatggtg agtaaccatg catcatcagg agtacggata	600
aaatgcttgc tggatcgaaag aggatataat tccgtcagcc agtttagtct gaccatctca	660
tctgtAACAT cattggcaac gctacccttgc ccatgtttca gaaacaactc tggcgcatcg	720
ggcttcccat acaatcgata gattgtcgca cctgattgcg cgacattatc gcgagccat	780
ttatacccat ataaatcgc atccatgttg gaatttaatc ggggcctcga gcaagacgtt	840
tcccgttggaa tatggctcat aacaccctt gtattactgt ttatgtaaac agacaggctcg	900
acaatattgg ctattggcca ttgcatacgatgtatctata tcataatatg tacattata	960
ttggctcatg tccaaatatga ccgcgcattttt gacattgtattt attgactgtt tattatgt	1020
aatcaattac ggggtcatta gttcatagcc catatatggaa gttccgcgtt acataactta	1080
cggtaaatgg cccgcctggc tgaccggcca acgacccccc cccattgcacg tcaataatga	1140
cgtatgttcc catagtaacg ccaataggaa ctttccatttgc acgtcaatgg gtggagtatt	1200
tacggtaaac tgcccacttg gcagtcacatc aagtgtatca tatgccaagt ccggccctta	1260
ttgacgtcaa tgacggtaaa tgccgcctt ggcattatgc ccagtcacatg accttacggg	1320
actttcttac ttggcagttac atctacgtat tagtcacatcg tattaccatg gtatgcgg	1380
tttggcagta caccaatggg cgtggatagc gggttgactc acggggatatt ccaagtcctcc	1440

-continued

accccatgatcgtaatggcgttttgcaccaaaa	tcaacgggac	tttccaaaat	1500
gtcgtaataaccggccccgttgacgcaaa	tggcggttag	gctgtacgg	1560
atataagcagagctcggtta	gtgaaccgctc	agatcgctcg	1620
ttgacacctatagaagacac	cgggaccgat	ccagcctccg	1680
gaacgcccgttcccgaaagatgtact	caccgtccgg	atctcagcaa	1740
actctccagggtggccctgg	cttcccagt	caagactcca	1800
ggcttcttcgttacatgtac	ctttgttttgc	cctcaacccct	1860
tcggcggatcttccatgtgttccatgt	gactatcttc	caggtcagga	1920
tgcgtccgaatttgcgttccatgttccatgt	aatctcccg	aggactgggg	1980
gaacatggcttgcgttccatgttccatgt	agcattgtgg	gaggctggga	2040
gtttgtggcccttcgttccatgttccatgt	gtgcgagaag	cattccaaac	2100
cctcacatgtgttccatgttccatgt	cggcggtgtt	ctgggtcacc	2160
gtttcatcttgcgttccatgttccatgt	cccagtgggt	ttggctgggc	2220
ctacgatatgttccatgttccatgt	agcctccatgt	agaatcgatt	2280
cctcatgtgttccatgttccatgt	cctcaggccaa	ggtgatgact	2340
cctgcccaccgttccatgttccatgt	caggagccat	cactggggac	2400
tgaaccagaggttccatgttccatgt	ccccaaagaa	acttcagtgt	2460
caatgacgtgttccatgttccatgt	ttcacccctca	gaaggtgacc	2520
acgctggacaatgttccatgttccatgt	ggggcaaaa	aaatcgatt	2580
tgggtgtcttgcgttccatgttccatgt	caaggtatca	cgtcatgggg	2640
ttccctgttacatgttccatgttccatgt	accaggatgg	tgcattaccg	2700
ccccggatccatgttccatgttccatgt	gaaggtaggg	gacctgtgg	2760
accgcgttgcgttccatgttccatgt	aaggctgtgc	tttgatggca	2820
cactgcctgtgttccatgttccatgt	cctgaaagc	ccaggtgagc	2880
ggagaactgcgttccatgttccatgt	acccagctgg	gggagcagtg	2940
cctgaccgttgcgttccatgttccatgt	atcagcaaag	gctcagtttgc	3000
cgtggcaagatgttccatgttccatgt	aagaacatca	cgtgtgttgc	3060
tgcgttccatgttccatgttccatgt	ccggcttgc	ccatccctgc	3120
gggaccggcgttccatgttccatgt	cagctaggat	cccagaccct	3180
tgtggaaagcatgttccatgttccatgt	aaccaggcc	caatggcaag	3240
ggcgctgggttgcgttccatgttccatgt	ctggcggttgc	cttcgggttgc	3300
atcctccatgttccatgttccatgt	gaagctacta	acattactcc	3360
attgaaagcttgcgttccatgttccatgt	agaagtttgc	aaagcataat	3420
aggaacagaaatgttccatgttccatgt	caaaaatttc	gcaatttgc	3480
cctggattcttgcgttccatgttccatgt	gttggatgttgc	cacattatga	3540
tcccaactacatctcaataatgttccatgt	ttaatgttgc	tggaaatgag	3600
tgaaccacccatgttccatgttccatgt	cctccaggat	atggaaatgttgc	3660
ctctccttcaatgttccatgttccatgt	ggaatgccag	agggcgatct	3720
cttctttaatgttccatgttccatgt	ttggAACGGG	acatgaaaat	3780

-continued

atatggaaa gtttcagag gaaataaggt	taaaaatgcc	cagctggcag	ggccaaagg	3840		
agtcatctc tactccgacc	ctgctgacta	cttgcctct	ggggtaagt	cctatccaga	3900	
tggttgaat cttctggag	gtggtgtcca	gcgtggaaat	atcctaaatc	tgaatggtgc	3960	
aggagaccct ctcacaccag	gttacccagc	aatgaatat	gcttataggc	gtgaaattgc	4020	
agaggctgtt	ggtcttccaa	gtattccgt	tcatccaatt	ggatactatg	atgcacagaa	4080
gctcctagaa	aaaatgggtg	gctcagcacc	accagatagc	agctggagag	gaagtctcaa	4140
agtgcctac	aatgttggac	ctggctttac	tggaaacttt	tctacacaaa	aagtcaagat	4200
gcacatccac	tctaccaatg	aagtgacaag	aatttacaat	gtgataggta	ctctcagagg	4260
agcagtggaa	ccagacagat	atgtcattct	gggaggtcac	cgggactcat	gggtgtttgg	4320
tggtattgac	cctcagagtg	gagcagctgt	tgttcatgaa	attgtgagga	gctttggAAC	4380
actgaaaaag	gaagggtgga	gacctagaag	aacaattttg	tttgcagact	gggatgcaga	4440
agaatttgg	cttcttgggtt	ctactgagtg	ggcagaggag	aattcaagac	tccttcaaga	4500
gcgtggcggt	gcttatattta	atgctgactc	atctatagaa	ggaaactaca	ctctgagagt	4560
tgattgtaca	ccgctgtatgt	acagcttgggt	acacaaccta	acaaaagagc	tgaaaagccc	4620
tgatgaaggc	tttgaaggca	aatctcttta	tgaaagtgg	actaaaaaaaa	gtccttcccc	4680
agagttcagt	ggcatgccc	ggataagcaa	attgggatct	ggaaatgatt	ttgaggttt	4740
cttccaacga	cttggattt	cttcaggcag	agcacggtat	actaaaaatt	gggaaacaaa	4800
caaattcagc	ggctatccac	tgtatcacag	tgtctatgaa	acatatgagt	tggggaaaa	4860
gttttatgt	ccaatgttta	aatatcacct	cactgtggcc	cagggtcgag	gagggatgg	4920
gtttgagctg	gccaattcca	tagtgcctcc	tttgattgt	cgagattatg	ctgtagttt	4980
aagaaaagtat	gctgacaaaa	tctacagtat	ttctatgaa	catccacagg	aatgaagac	5040
atacagtgt	tcatttgatt	cactttttc	tgcagtaaa	aattttacag	aaattgcctc	5100
caagttcagt	gagagactcc	aggactttga	caaaagcaac	ccaatagtat	taagaatgat	5160
gaatgatcaa	ctcatgttcc	tggaaagagc	atttattgt	ccattagggt	taccagacag	5220
gccttttat	aggcatgtca	tctatgctcc	aagcagccac	aacaagtatg	cagggagtc	5280
atccccagga	atttatgtat	ctctgtttga	tattgaaagc	aaagtggacc	cttccaaggc	5340
ctggggagaa	gtgaagagac	agatttatgt	tgcagccctc	acagtcgagg	cagctgcaga	5400
gactttgagt	gaagtagcct	aaagatctgg	gccctaaca	aacaaaaga	tggggttatt	5460
ccctaaactt	catgggttac	gtaattggaa	gttggggac	attgccacaa	gatcatattg	5520
tacaaaagat	caaacactgt	tttagaaaac	ttctgtaaa	caggcctatt	gattggaaag	5580
tatgtcaaaag	gattgtgggt	cttttgggt	ttgctgctcc	atttacacaa	tgtggatatc	5640
ctgccttaat	gccttgtat	gcatgtatac	aagctaaaca	ggcttcact	ttctcgccaa	5700
cttacaaggc	ctttctaaat	aaacagtaca	tgaaccttta	ccccgtgt	cggcaacggc	5760
ctggctgtgt	ccaagtgtt	gctgacgca	ccccactgg	ctggggctg	gccataggcc	5820
atcagcgcac	gcgtggacc	tttgggtctc	ctctgccc	ccatactgcg	gaactcctag	5880
ccgcttggtt	tgctcgcagc	cggctctggag	caaagctcat	aggaactgac	aattctgtcg	5940
tcctctcgcg	gaaatataca	tcgtttcgat	ctacgtatga	tcttttccc	tctgcca	6000
attatggggaa	catcatgaag	ccccttgagc	atctgacttc	tggctataaa	aggaaattta	6060
tttcattgc	aatagtgtgt	tggattttt	tgtgtctctc	actcgaaagg	aattctgcat	6120
taatgaatcg	gccaacgcgc	ggggagagggc	ggtttgcgt	ttggggcgctc	ttccgctcc	6180

-continued

tcgctcactg actcgctgcg ctcggcggtt cggtcgcc gaggatcactc agtcactca	6240
aaggcgtaa tacgggttac cacagaatca gggataacg caggaaagaa catgtgagca	6300
aaaggccagc aaaaggccag gaaccgtaaa aaggccgcgt tgctggcggtt tttccatagg	6360
ctccggcccc ctgacgagca tcacaaaaat cgacgctcaa gtcagagggtg gcgaaacccg	6420
acaggactat aaagatacca ggcgtttccc cctggaagct ccctcgctgctt ctctccgtt	6480
ccgaccctgc cgcttacccgg atacctgtcc gccttttccctt ccgtggaaag cgtggcgctt	6540
tctcatagct cacgtgttagt gtatctcagt tgggtgttagg tgggtcgctc caagctggc	6600
tgtgtgcacg aaccccccgt tcagccgcac cgctgcgcct tatccggtaa ctatcgctt	6660
gagtccaaacc cggtaagaca cgacttacg ccactggcag cagccactgg taacaggatt	6720
agcagagcga ggtatgttagg cgggtctaca gagttcttga agtgggtggcc taactacggc	6780
tacactagaa gaacagtatt tggtatctgc gctctgctga agccagttac ctccggaaaa	6840
agagttggta gcttctgatc cggcaaaaca accaccgttg ttagccgggttgg ttttttgg	6900
tgcaaggcagc agattacgcg cagaaaaaaa ggatctcaag aagatccccc gatctttct	6960
acggggctctg acgctcagtg gaacggaaac tcacgttaag ggattttggg catgagatta	7020
tcaaaaagga tttcaccta gatcccttta aattaaaaat gaagttttaa atcaatctaa	7080
agtatatatg agtaaaacttg gtcgacagt taccatgtc taatcgtga ggcacccatc	7140
tcagcgatct gtctatccgtt ttcatccata gttgcctgac tc	7182

<210> SEQ ID NO 36

<211> LENGTH: 7694

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 36

ggcgtaatgc tctggcagtg ttacaacca ttaaccaatt ctgatttagaa aaactcatcg	60
agcatcaaat gaaactgcaat ttatttcata tcaggattat caataccata tttttggaaaa	120
agccgtttct gtaatgaagg agaaaactca ccgaggcagt tccataggat ggcaagatcc	180
tggtatcggt ctgcgattcc gactcgatca acatcaatac aacctattaa tttccctcg	240
tcaaaaataa ggttatcaag tgagaaatca ccatgagtga cgactgaatc cggtgagaat	300
ggcaaaaactt tgcattttc ttccagact tggtaacac ggcagccatt acgctcgatca	360
tcaaaaatcac tcgcatcaac caaacgtta ttcatcgat attgcgcctg agcgagacga	420
aatacgcgtt cgctgttaaa aggacaatta caaacaggaa tcaaataatgca cccggcgcagg	480
aacactgcca ggcgcataac aatattttca cctgaatcag gatattcttc taatacctgg	540
aatgcgtttt tcccgggat cgcagttggt agtaaccatg catcatcagg agtacggata	600
aaatgcttgc tggtcggaaag aggatataat tccgtcagcc agtttagtct gaccatctca	660
tctgtacat cattggcaac gctacccctt ccatgtttca gaaacaactc tggcgcatcg	720
ggcttcocat acaatcgata gattgtcgca cctgattgcc cgacattatc gcgagccat	780
ttatacccat ataaatcagc atccatgtt gaaatataatc gcccgcctcg gcaagacgtt	840
tcccggttggaa tatggctcat aacacccttt gtattactgt ttatgtaaac agacaggctcg	900
acaatattggt tattggcca ttgcatacgat tggatctata tcataatatg tacatttata	960
ttggctcatg tccaaatatgca cccgcattttt gacattgtt attgactagt tattaaatgt	1020

US 9,468,672 B2

299**300**

-continued

aatcaattac	ggggtcatta	gttcatagcc	catatatgga	gttccgcgtt	acataactta	1080
cggtaaatgg	cccgcctggc	tgaccgcccc	acgacccccc	cccattgacg	tcaataatga	1140
cgtatgttcc	catagtaacg	ccaataggga	cttccatttgc	acgtcaatgg	gtggagtatt	1200
tacggtaaac	tgcccacttg	gcagttacatc	aagtgtatca	tatgccaagt	ccgcccccta	1260
ttgacgtcaa	tgacggtaaa	tgccccgcgt	ggcatttatgc	ccagtgatcg	accttacggg	1320
acttcttac	ttggcagttac	atctacgtat	tagtcatcg	tattaccatg	gtgtgcgg	1380
tttggcagta	caccaatggg	cgtggatagc	ggtttgactc	acggggattt	ccaagtctcc	1440
accccattga	cgtcaatggg	agtttggttt	ggcacaaaaa	tcaacgggac	tttccaaaat	1500
gtcgtataaa	ccccgcctcc	ttgacgcataa	ttggccgttag	gcgtgtacgg	tgggaggtct	1560
atataagcg	agctcgttt	gtgacccgtc	agatcgcc	gagacgc	ccacgcgttt	1620
ttgaccccca	tagaagacac	cgggaccgat	ccagccctcg	cggccgggaa	cggcgtattt	1680
gaacgcggat	tccccgtgcc	aagagtgtact	caccgtccgg	atctcagcaa	gcaggtatgt	1740
actctccagg	gtggggctgg	cttcccccagt	caagactcca	gggatttgag	ggacgcgttg	1800
ggcttcttc	ttacatgtac	ctttgtcttgc	cctcaacccct	gactatcttc	caggtcagga	1860
tcccagagtc	aggggtctgt	atttccttgc	ttgtggctcc	agttcaggaa	cagtaaaccc	1920
tgctccgaat	attgcctctc	acatctcg	aatctcccg	aggactgggg	accctgtgac	1980
gaacatggct	agcaaggctg	tgctgcttgc	cctgttgc	gcaggcttgg	ccctgcagcc	2040
aggcactgcc	ctgctgtgt	actctcgaa	agcccagggt	agcaacgagg	actgcctgca	2100
ggtggagaac	tgcacccagc	tgggggagca	gtgctggacc	gcgcgcatcc	gcgcagttgg	2160
cctcctgacc	gtcatcagca	aaggctcg	cttgaactgc	gtggatgact	cacaggacta	2220
ctacgtggc	aagaagaaca	tcacgtgt	tgacaccgac	ttgtgcac	ccagcggggc	2280
ccatgcctg	cagccggctg	ccgcacatct	tgcgtgt	cctgcactcg	gcctgtgt	2340
ctggggaccc	ggccagctag	gatcccagac	cctgaacttt	gatctgtga	aactggcagg	2400
cgtatgtggaa	agcaacccag	gcccaatggc	aagcgcgc	cgccccgcgt	ggctgtgcgc	2460
tggggcgctg	gtgctggcgg	gtgggttctt	tctcctcg	tccctttcg	gtgtgtttat	2520
aaaatcctcc	aatgaagcta	ctaacattac	tccaaagcat	aatatgaaag	catttttgg	2580
tgaattgaaa	gctgagaaca	tcaagaagtt	cttatataat	tttacacaga	taccacattt	2640
agcaggaaca	gaacaaaact	ttcagcttgc	aaagcaaatt	caatcccagt	ggaaagaatt	2700
tggcctggat	tctgttgagc	tggcacatta	tgtatgtcttgc	ttgtcc	caaataagac	2760
tcatccaaac	tacatctcaa	taattatga	agatggaaat	gagatttca	acacatcatt	2820
atttgaacca	cctcctccag	gatatgaaaa	tgttccggat	attgtaccac	ctttcagtgc	2880
tttctcttct	caaggaatgc	cagagggcga	tctagtgtat	gttaactatg	cacgaactga	2940
agacttcttt	aaatttggaaac	ggggacatgaa	aatcaattgc	tctggggaaa	ttgtatgc	3000
cagatatggg	aaagtttca	gaggaaataa	ggttaaaaat	gcccgactgg	caggggc	3060
aggagtctt	ctctactccg	accctgtga	ctactttgc	cctgggggtga	agtcctatcc	3120
agatgggttgg	aatcttcctg	gaggtgggt	ccagcgtgga	aatatcctaa	atctgaatgg	3180
tgcaggagac	cctctcacac	caggttaccc	agcaaatgaa	tatgcttata	ggcgtggaaat	3240
tgcagaggct	gttggcttcc	caagtattcc	tgttcatcc	atggataact	atgtgcaca	3300
gaagctccta	aaaaaaatgg	gtggctcagc	accaccagat	agcagctgga	gaggaagtct	3360
caaagtcccc	tacaatgttg	gacctggctt	tactggaaac	ttttctacac	aaaaagtcaa	3420

-continued

gatgcacatc cactctacca atgaagtgac aagaatttac aatgtgatag gtactctcag	3480
aggagcagtg gaaccagaca gatatgtcat tctgggaggt cacccggact catgggttgtt	3540
tggtgttatt gaccctcaga gtggagcagc tggtgttcat gaaattgtga ggagcttgg	3600
aacactgaaa aaggaaagggt ggagacctag aagaacaatt ttgtttgcaa gctggatgc	3660
agaagaattt ggtcttcttg gttctactga gtgggcagag gagaattcaa gactcctca	3720
agagcgtggc gtggcttata ttaatgctga ctcatctata gaaggaaact acactctgag	3780
agttgattgt acaccgctga tgtacagtt ggtacacaac ctaacaaaag agctgaaaag	3840
cctctgatgaa ggcttgaag gcaaatctct ttatgaaagt tggactaaaa aaagtccccc	3900
cccagagttc agtggcatgc ccaggataag caaattggga tctggaaatg atttttaggt	3960
gttctccaa cgacttggaa ttgcttcagg cagagcacgg tatactaaaa attggaaac	4020
aaacaaattc agcggctatc cactgtatca cagtgcttat gaaacatatg agttggtgga	4080
aaagttttat gatcaatgt ttaaatatca cctcaactgtg gcccgaggtc gaggaggat	4140
ggtgttttagt otggccaatt ccatagtgtt ccctttgtat tgctcgagatt atgctgttagt	4200
tttaagaaag tatgtgtgaca aaatctacag tatttctatg aaacatccac aggaaatgaa	4260
gacatacagt gtatcattt attcaactttt ttctgcagta aagaattta cagaaattgc	4320
ttccaagttc agtgagagac tccaggactt tgacaaaagc aacccatag tattaagaat	4380
gatgaatgat caactcatgt ttctggaaag agcatttatt gatccattag gtttaccaga	4440
caggccttt tataggcatg tcacttatgc tccaaggcgc cacaacaagt atgcagggg	4500
gtcatttcca ggaattttatg atgetctgtt tgatattgaa agcaaagtgg acccttccaa	4560
ggcctggggaa gaagtgaaga gacagattt tggtgcagcc ttccacagtgc aggagctgc	4620
agagactttg agtgaagtag cctaaagatc tgacccctta acgttactgg ccgaagccgc	4680
tttggataag gccgggtgtc gtttgcata atgttattttt ccaccatatt gccgtcttt	4740
ggcaatgtga gggccggaa acctggccct gtcttctga cgagcattcc taggggtctt	4800
tccccctctcg ccaaaggaat gcaaggctcg ttgaatgtcg tgaaggaaagc agtccctctg	4860
gaagcttctt gaagacaaac aacgtctgtt gacccctttt gcaggcagcg gaaccccca	4920
cctgggacca ggtgectctg cggccaaaag ccacgtgtat aagatacacc tgcaaaaggcg	4980
gcacaacccc agtgcacacgt tgtgagttgg atagttgtgg aaagagtcaa atggctctcc	5040
tcaagcgtat tcaacaagggt gctgaaggat gcccagaagg tacccatgt tatggatct	5100
gatctggggc ctcggtgac atgcttaca tgggttttagt cgaggtaaa aaacgtctag	5160
gccccccgaa ccacggggac gtgggtttcc tttgaaaaac acgtatgataa tatggccagc	5220
attgtggag gctggagtg cgagaagcat tcccaacccctt ggcagggtgt tggccctct	5280
cgtggcaggg cagtcgtcgg cgggtttctg gtgcacccccc agtgggtcct cacagctgcc	5340
cactgcatca ggaacaaaag cgtgatcttg ctgggtcggc acagcttgc ttcatctgaa	5400
gacacaggcc aggtatttca ggtcagccac agttcccac acccgctcta cgatatgagc	5460
ctccctgaaga atcgatttcc tggccaggt gatgactcca gocacgaccc catgtgtc	5520
cgcctgtcag agcctgcccga gctcacggat gctgtgaagg tcatggactt gcccaccag	5580
gagccagcac tggggaccac ctgctacgcc tcaggctggg gcagcattga accagaggag	5640
ttcttgaccc caaagaaact tcagtgtgt gacctccatg ttatccaa tgacgtgtgt	5700
gcgcagttc accctcagaa ggtgaccaag ttcatgtgtt gtgctggacg ctggacaggg	5760

-continued

ggcaaaagca cctgtcgaaa tgattctggg ggcccaacttg tctgtatgg tgtgtttcaa	5820
ggtatcacgt catggggcag tgaaccatgt gcccgtcccc aaaggccttc cctgtacacc	5880
aagggtggtgc attaccggaa gtggatcaag gacaccatcg tggccaaccc ctgaggatct	5940
ggggccctaac aaaacaaaaa gatggggta ttccctaaac ttcatgggtt acgtaatgg	6000
aagttggggg acattgccac aagatcatat tgtacaaaag atcaaacact gtttagaaa	6060
acttcctgt aacaggccta ttgattggaa agtatgtcaa aggattgtgg gtctttggg	6120
ctttgtgtc ccatttacac aatgtggata tcctgcctta atgcctttgt atgcacgttat	6180
acaagctaaa caggcttca ctttcgtcc aacttacaag gccttctaa gttaaacatgt	6240
catgaacctt taccccggtt ctcggcaacg gcctggctcg tgccaagtgt ttgctgacgc	6300
aaccccccact ggctggggct tggccatagg ccatcagcgc atgcgtggaa ccttggc	6360
tcctctgccc atccatactg cgaaactctt agccgcttgtt ttgctcgca gccggctctgg	6420
agcaaaagctc ataggaactg acaattctgt cgttctctcg cggaaatata catcggttc	6480
atctacgtat gatcttttc cctctgcca aaattatggg gacatcatga agcccttga	6540
gcatctgact tctggctaat aaaggaaatt tattttcatt gcaatagtgtt gttggattt	6600
tttgggtctc tcactcggaa ggaattctgc attaatgaat cggcaacgc gcggggagag	6660
gcgggttgcg tattgggcgc tcttcgcctt cctcgctcac tgactcgctg cgctcggtcg	6720
ttcggctgcg gcgagcggta tcagctact caaaggcggt aatacgggtt tccacagaat	6780
caggggataa cgcaggaaag aacatgtgag caaaaggcca gaaaaaggcc aggaaccgt	6840
aaaaggccgc gttgctggcg ttttccata ggctccgccc ccctgacgag catcacaaaa	6900
atcgacgctc aagtcaagg tggcgaaacc cgacaggact ataaagatac caggcgttc	6960
cccccgttgcg ttttccatcg ttttccatcg ttttccatcg ttttccatcg ttttccatcg	7020
ccgcctttct cccttcggga agcgtggcgc ttttccatcg ttttccatcg ttttccatcg	7080
gttcgggtgtt ggtcggtcg tccaaatcg ttttccatcg ttttccatcg ttttccatcg	7140
accgctgcgc ttttccatcg ttttccatcg ttttccatcg ttttccatcg ttttccatcg	7200
cggcactggc agcagccact ggtaacagga ttagcagacg gaggtatgtt ggcgggtgtt	7260
cagagttctt gaagtgggtt cctaaatcg ttttccatcg ttttccatcg ttttccatcg	7320
ggcgtctgtt gaagccagtt accttcggaa aaagagttgg tagtcttgc tccggcaac	7380
aaaccaccgc tggtagcggt ggttttttgc ttttccatcg ttttccatcg ttttccatcg	7440
aaggatctca agaagatcct ttgatctttt ctacggggcgt tgacgtcg tggaaacgaaa	7500
actcacgtt aaggatctt gtcgtcgat tatcaaaaag gatcttccacc tagatccctt	7560
taaaataaaa atgaagttttt aatcaatct aaagtatata tggtaaaact tggtctgaca	7620
gttaccaatg ottaatcgt gaggcaccta tctcagcgt ctgtctattt cggtcatcca	7680
tagttgcctg actc	7694

<210> SEQ ID NO 37

<211> LENGTH: 8461

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 37

catcatcaat aatacacctt atttggatt gaagccaata tgataatgag ggggtggagt	60
ttgtgacgtg gcgccccgg tgggaacggg gcgggtgacg tagtagtgtg gcggaaatgt	120

-continued

gatgttgc aa gtgtggcg ga acacatgt aa gcgacggat g tggcaaaagt gacgttttg	180
gtgtgcgc gg gtgtacac ag gaagtgc aa tttcgcgc gttttagggc gatgtttag	240
taaattggg cgtaaccgag taagatttgg ccatttcgc gggaaaactg aataagagga	300
agtgaatctt gaataatttt gtgttactca tagcgcgtaa tactgtaaata gtaatcaatt	360
acggggtcat tagttcatag cccatatatg gagttccgcg ttacataact tacggtaat	420
ggccgcctg gctgaccgc caacgacccc cgccattga cgtcaataat gacgtatgtt	480
cccatagtaa cgccaatagg gactttccat tgacgtcaat gggtggagta tttacggtaa	540
actgcccact tggcagttaca tcaagtgtat catatgc caa gtacgc ccc tattgacgtc	600
aatgacggta aatggccgc ctggcattat gcccagttaca tgaccttatg ggactttcct	660
acttggcagt acatctacgt attagtcatc gctattacca tggtgatgcg gttttggcag	720
tacatcaatg ggcgtggata gcggtttgac tcacggggat ttccaagttt ccacccatt	780
gacgtcaatg ggagttgtt ttggcaccaa aatcaacggg actttccaaa atgtcgtaac	840
aactccgc cattgacgc aatgggcggt aggctgtac ggtgggaggt ctatataagc	900
agagctgggtt tagtgaaccg tcagatccgc tagagatcca ccatggctag cggtgcccg	960
acgttgc ccc ctgcctggca gccc ttctc aaggaccacc gcatcttac attcaagaac	1020
tggcccttct tggagggtc cgcctgcgc cccgggggg tggccgaggc tggcttcatc	1080
cactgeccca ctgagaacga gccagacttgc gcccagtgtt tttctgctt caaggagctg	1140
gaaggctggg agccagatga cgacccata gaggaacata aaaagcatc gtccgggtgc	1200
gttttcttt ctgtcaagaa gcagtttgaat gatataaccc ttggtaatt tttgaaactg	1260
gacagagaaa gagccaagaa caaaaattca aaggaaacca acaataagaa gaaagaattt	1320
gaggaaactg cggagaaagt gcgcgcgtgc atcgagcgc tggctgcacat ggattagaga	1380
tctgaccccc taacgttact ggccgaagec gcttggata aggccgggtgt gcgttgc	1440
atatgttatt ttccaccata ttgcgcgtt ttggcaatgt gaggggccgg aaacctggcc	1500
ctgtcttctt gacgagcatt cctagggtc ttccctctt cgcacaaagg atgcaaggc	1560
tgttgaatgt cgtgaaggaa gcagtttctc tggaaagacaa acaacgtctg	1620
tagcgaccct ttgcaggcag cggacccccc cacctggcga caggtgcctc tgccggccaa	1680
agccacgtgt ataagataca cctgcaaaagg cggcacaacc ccagtgcac gttgtgagtt	1740
ggatagttgtt ggaaagagtc aatggctct cctcaagcgtt attcaacaag gggctgaagg	1800
atgcccagaa ggtacccat tttatggat ctgtatgggg gcctcggtgc acatgttta	1860
catgtgttta gtcgagggtt aaaaacgtct agggccccc aaccacgggg acgtggttt	1920
cctttgaaaa acacgataat atggccgc ctcgagccta agcttctaga taagatatcc	1980
gatccacccg atctagataa ctgtatcataa tcagccatac cacattgtt gaggtttac	2040
ttgctttaaa aaacctcccc cacctcccc tgaacctgaa acataaaatg aatgcattt	2100
ttgttgtttaa cttgtttattt gcagttataa atggttacaa ataaagcaat agcatcacaa	2160
atttcacaaa taaagcattt ttttactgc attctagttt tggtttgc aaactcatca	2220
atgttatcttta acgcggatct gggcgtgggtt aagggtggga aagaatataat aagggtgggg	2280
tctttagttatct ttttgcgcg acgcgcgc gccatgagca ccaactcgat	2340
tgtatggaaacg attgtgagct catatttgc aacgcgcatg ccccatggg ccgggggtgc	2400
tcagaatgttgc atgggcgttca gcattgttgc tgcggccgc tgcggccaa actctactac	2460

-continued

cttgacctac gagaccgtgt ctggaacgcc gttggagact gcagcctcg ccgcgccttc	2520
agccgcgtca gccaccgccc gcgggattgt gactgacttt gcttctcga gcccgcgtgc	2580
aaggcagtgc a gcttcccggtt c atccgccccg cgatgacaag ttgacggctc ttttggcaca	2640
atggattct ttgaccgggg aacttaatgt cgtttctcag cagctgttgg atctgcgcca	2700
gcagggttct gcccgtaaagg ctccctcccc tcccaatgcg gtttaaaaca taaaataaaaa	2760
accagactct gtttggattt ggtcaagca agtgtctgc tgtctttttaggggttt	2820
g cgcgcgcgg taggccccggg accagcggtc tcggtcgttgg agggtcctgt gtatttttc	2880
caggacgtgg taaagggtac tctggatgtt cagatacatg ggcataagcc cgtctctggg	2940
gtggaggtag caccactgca gagcttcatg ctgcgggggtg gtgtttaga tgatccagtc	3000
gttagcaggag cgctggcggt ggtgcctaaa aatgtctttc agtagcaagc tgattgcag	3060
gggcaggccc ttgggttaag tttttacaaa g cgggttaagc tgggatgggt gcatacgtgg	3120
ggatatgaga tgcacatctgg actgtatTTT taggttggct atgttccacccatccat	3180
c cggggatTC atgttgcacca g aaccaccag cacagtgtat cccgtgcact tggaaattt	3240
gtcatgtacg ttagaggaa atgcgtggaa gaaacttggag acgccttgcgt gaccccaag	3300
atTTTccatg cattcgtcca taatgtatggc aatggggccca cggggccggg cctggggcga	3360
gatatttctg ggatcactaa cgtcatagtt gtgttccagg atgagatcgt cataggccat	3420
ttttacaaag cgcggccggg ggggtccaga ctgcggata atggttccat cccggccagg	3480
ggcgttagtta ccctcacaga tttgcatttcc acgcgtttt agttcagatg gggggatcat	3540
gtctacctgc ggggcgtatga agaaaacgggt tttccgggtt ggggagatca gctgggaaga	3600
aagcagggttc ctgagcagct gcgacttacc gcagccgggtg gcccgtaaa tcacacctat	3660
tacccgggtgc aactggtagt taagagatgt gcagctgcgc tcacccctga gcaggggggc	3720
cacttcgtta agcatgtccc tgactcgcat gtttccctg accaaatccg ccagaaggcg	3780
ctcgccccc agcgatagca gtttctgca ggaagcaag ttttcaacg gtttggagacc	3840
gtccggcgtta ggcacgttt tgagcgtttt accaaggcgtt tccaggcgtt cccacagctc	3900
ggtcacactgc tctacggcat ctgcgtccat ccatatctcct cgtttcgcgg gttttggcg	3960
cttgcgtgt acggcgttagt tcgggtctcg tccagacggg ccagggtcat gtctttcac	4020
gggcgcgggg tccctcgtagt cgttagtctgg gtcacgggtga aggggtgcgc tccgggtgc	4080
gcgcgtggcca ggggtcgctt gaggctggtc ctgcgtgtc tgaagcgtg cccgtctcg	4140
cctcgcgcgtt cggccaggta gcatggacc atgggtgtcat agtccacccc ctccgcggcg	4200
tggcccttgg cgcgcagctt gcccctggag gaggcgcggc acggggggca gtgcagactt	4260
ttgagggcgtt agagcttggg cgcgcgaaat accgatccg gggagtaggc atccgcggc	4320
caggccccgc agacgggttc gcatggacc agccagggtga gtcctggccg ttcggggtca	4380
aaaaccagggtt tccccccatg cttttgtat cgtttcttac ctctggtttccatggccgg	4440
tgtccacgctt cgggtacgaa aaggctgtcc gttttccgtt atacagactt gagagggagt	4500
ttaaacaaat tcaatagctt gttgcatggg cggcgatata aatgcacgg tgctgctaa	4560
aaaatcaggc a aaggcctcgc gcaaaaaaaga aagcacatcg tagtcatgct catgcagata	4620
aaggcaggta agctccggaa ccaccacaga aaaagacacc attttctct caaacatgtc	4680
tgccgggttc tgcataaaaca caaaaataaaa taacaaaaaa acattnaac attagaagcc	4740
tgtcttacaa caggaaaaac aacccttata agcataagac ggactacggc catgcggcg	4800
tgaccgtaaa aaaactggtc accgtgatta aaaagcacca cccacagctc ctgcgtcatg	4860

-continued

tcggaggatca taatgttaaga ctccggtaaac acatcaggtt gattcacatc ggtcagtgc 4920
 aaaaaggcgac cgaaatagcc cgggggataa cataccccga ggcgttagaga caacattaca 4980
 gccccatag gagggtataac aaaattaata ggagagaaaa acacataaac acctgaaaaa 5040
 ccctcctgcc taggcaaaaat agcaccctcc cgctccagaa caacatacag cgcttccaca 5100
 gccggcggca taacagtcag ccttaccagt aaaaaagaaa accttattaa aaaacaccac 5160
 tcgacacggc accagctcaa tcagtcacag tgaaaaaaaaa ggccaagtgc agagcgagta 5220
 tatataggac taaaaaatga cgtAACCGTT aaagtccaca aaaaacaccc agaaaacccgc 5280
 acgcgaacct acgcccagaa acgaaagcca aaaaacccac aacttcctca aatcgtaact 5340
 tcggtttcc cacgttacgt cacttcccat tttaagaaaa ctacaattcc caacacatac 5400
 aagttactcc gccctaaaac ctacgtcacc cgcgggttc ccacgccccg cgccacgtca 5460
 caaactccac cccctcatta tcataattggc ttcaatccaa aataaggtat attattgtg 5520
 atgttaatta acatgtcatgg atccatatgc ggtgtgaaat accgcacaga tgcgtaaagga 5580
 gaaaataccg catcaggcgc tcttccgctt cctcgctcac tgactcgctg cgctcggtcg 5640
 ttccggctcgcc gcgagcggtt tcagtcact caaaggcggtt aatacggtt tccacagaat 5700
 caggggataa ogcaggaaag aacatgttag caaaaggcca gaaaaaggcc aggaaccgtt 5760
 aaaaaggccgc gttgtggcg ttttccata ggctccgc ccctgacgag catcacaaaaa 5820
 atcgaegectc aagtcaagagg tggtggaaacc cgacaggact ataaagatac caggegtt 5880
 cccctggaaat ctccctcggt cgctctccgtt ttccgaccct ggccgttacc ggataacctgt 5940
 ccgcctttctt cccttcggga agcgtggcgc ttctcatag ctcacgttgtt aggtatctca 6000
 gttccgggttgc ggtcggttgc tccaaagctgg gctgtgtgcga cgaacccccc gttcagcccg 6060
 accgctgcgc cttatccggt aactatcgcc ttgagtcctt cccggtaaga cacgacttat 6120
 cgccactggc agcagccact ggtaacacgga ttagcagagc gaggtatgtt ggcggcgta 6180
 cagagttttttaa gaagttgggg ccttaactacgt gctacacttag aaggacagta tttggatct 6240
 ggcgtctgtt gtggccatgtt accttcggaa aaagagtgg tagcttttgc tccggcaac 6300
 aaaccaccgc tggtagcggtt ggttttttgc tttgcaagca gcagattacg cgcagaaaaa 6360
 aaggatctca agaagatctt ttgatctttt ctacggggtc tgacgtcgat tggAACGAAA 6420
 actcacgttta agggattttgc tgcgttgat tatcaaaaag gatcttcacc tagatcctt 6480
 taaaataaaaa atgaagttttttaa aatcaatctt aaagttatata tggatcttgc tggatctgaca 6540
 gttaccaatgtt cttatctgtt gaggcaccta tctcagcgat ctgtcttgc tggatcttgc 6600
 tagttgcctgtt actcccccgtt gttgttgatata ctacgatacg ggagggttccatccatcc 6660
 ccagtgctgc aatgataccg cgagacccac gtcacccgc tccagattta tcagcaataa 6720
 accagccagc cggaaaggcc gggcgccatggaa gtggccctgc aactttatcc gcctccatcc 6780
 agtcttattaa ttgttgcggg gaagcttagag taagtttttgc ggcagttaaat agtttgcgca 6840
 acgttggtc cattgttgca gccatggat tatcaaaaag gatcttcacc tagatcctt 6900
 tcaacgttggaa agccaggccatgg cggaaacccgtt gctgacccttccatccatcc 6960
 ctatctggac aaggaaaaac gcaaggccaa agagaaaaac ggttagcttgc agtggccatcc 7020
 catggccatggatggccatgg gggccatggatggccatgg gggccatggatggccatggatggccatgg 7080
 gggccatggatggccatggatggccatggatggccatggatggccatggatggccatggatggccatgg 7140
 caaggatctg atggccatggatggccatggatggccatggatggccatggatggccatggatggccatgg 7200

-continued

gcatgattga acaagatgga ttgcacgcag gttctccggc cgcttgggtg gagaggctat 7260
 tggctatga ctggcacaa cagacaatcg gctgctctga tgccgcgtg ttccggctgt 7320
 cagcgcaggg gcgcgcggg cttttgtca agaccgacct gtccggtgcc ctgaatgaac 7380
 tgcaagacga ggcagcgcgg ctatcgtggc tggccacgac gggcgttcct tgccagctg 7440
 tgctcgacgt tgcactgaa gcggaaaggg actggctgct attggcgaa gtgcggggc 7500
 aggatctctt gtcatctcac cttgtctgtt ccgagaaaatg atccatcatg gctgtgcaa 7560
 tgccggggct gcatacgctt gatccggcta cctgcccatt cgaccaccaa gcgaaacatc 7620
 gcatcgagcg agcaactact cgatggaaag ccggcttgcgatcaggat gatctggacg 7680
 aagagcatca ggggctcgcc cgagccgaaac tgttcgccag gctcaaggcg agcatgccc 7740
 acggcgagga tctcgctgtt accatggcg atgcctgtt gccaatatac atggtgaaa 7800
 atggccgctt ttctggattc atgcactgtg gccggctggg tgtggcgac cgctatcagg 7860
 acatagcggtt ggctacccgtt gatattgtcg aagagcttgg cggcgaatgg gctgaccgct 7920
 tcctcggtctt tacggattc gcccgtcccg attcgacgcg catcgcccttc tatcgcccttc 7980
 ttgacgagtt cttctgaatt ttgttaaaat tttgttaaaat ttagctcatt tttaaccaa 8040
 taggcccggaaat tcggcaccat cccttataaa tcaaaagaat agaccgagat agggttgagt 8100
 gttgttccag tttggaaacaa gagtccacta ttaaagaacg tggactccaa cgtcaagg 8160
 cggaaaaacccg tctatcaggcgatggccca ctacgtgaaac catcaccctt atcaagttt 8220
 ttgtgtcgaaat ggtggccgtaa agcactaaat cggAACCTTA aaggggagccc ccgatttaga 8280
 gcttgcggg gaaagccggc gaacgtggcg agaaaggaaag ggaagaaagc gaaaggagcg 8340
 ggccgttaggg cgctggcaag tgtagcggtc acgctgcgcg taaccaccac acccgccgc 8400
 ttaatgcgcc gctacaggcgc gcttccattt gccattcagg atcgaattaa ttcttaattt 8460
 a 8461

<210> SEQ_ID NO 38

<211> LENGTH: 956

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 38

Met	Ala	Ser	Glu	Lle	Ala	Ala	Lle	Cys	Arg	Trp	Gly	Lle	Lle	Lle	Ala
1			5				10					15			

Lle	Lle	Pro	Pro	Gly	Ala	Ala	Ser	Thr	Gln	Val	Cys	Thr	Gly	Thr	Asp
20				25							30				

Met	Lys	Lle	Arg	Lle	Pro	Ala	Ser	Pro	Glu	Thr	His	Lle	Asp	Met	Lle
35				40							45				

Arg	His	Lle	Tyr	Gln	Gly	Cys	Gln	Val	Val	Gln	Gly	Asn	Lle	Glu	Lle
50				55							60				

Thr	Tyr	Lle	Pro	Thr	Asn	Ala	Ser	Lle	Ser	Phe	Lle	Gln	Asp	Ile	Gln
65				70							75			80	

Glu	Val	Gln	Gly	Tyr	Val	Lle	Ile	Ala	His	Asn	Gln	Val	Arg	Gln	Val
85				90							95				

Pro	Lle	Gln	Arg	Lle	Arg	Ile	Val	Arg	Gly	Thr	Gln	Lle	Phe	Glu	Asp
100				105							110				

Asn	Tyr	Ala	Lle	Ala	Val	Lle	Asp	Asn	Gly	Asp	Pro	Lle	Asp	Ser	Val
115				120							125				

Ala	Pro	Ala	Ala	Gly	Ala	Thr	Pro	Gly	Gly	Lle	Gln	Glu	Lle	Gln	Lle
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

-continued

130	135	140
Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val	Leu Ile Arg Arg Ser	
145	150	155
160		
Pro Gln Leu Cys His Gln Asp Thr Val	Leu Trp Glu Asp Val Phe Arg	
165	170	175
Lys Asn Asn Gln Leu Ala Leu Val	Leu Met Asp Thr Asn Arg Ser Arg	
180	185	190
Ala Cys His Pro Cys Ala Pro Met Cys Lys Ala Asn His Cys Trp Gly		
195	200	205
Glu Ser Ser Gln Asp Cys Gln Thr Leu Thr Arg Thr Ile Cys Thr Ser		
210	215	220
Ala Cys Ala Arg Cys Lys Ala Pro Leu Pro Thr Asp Cys Cys His Glu		
225	230	235
240		
Gln Cys Ala Ala Gly Cys Thr Gly Pro	Lys His Ser Asp Cys Leu Ala	
245	250	255
Cys Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala		
260	265	270
Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu		
275	280	285
Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn		
290	295	300
Tyr Leu Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His		
305	310	315
320		
Asn Gln Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys		
325	330	335
Ser Lys Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu		
340	345	350
Arg Glu Ala Arg Ala Ile Thr Ser Ala Asn Val Gln Asp Phe Val Gly		
355	360	365
Cys Lys Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp		
370	375	380
Gly Asp Pro Ala Ser Gly Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln		
385	390	395
400		
Val Phe Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala		
405	410	415
Trp Pro Asp Ser Phe Pro Asn Leu Ser Val Phe Gln Asn Leu Arg Val		
420	425	430
Ile Arg Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln		
435	440	445
Gly Leu Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Gln Glu Leu Gly		
450	455	460
Ser Gly Leu Ala Leu Val His Arg Asn Ala Arg Leu Cys Phe Val His		
465	470	475
480		
Thr Val Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu		
485	490	495
His Ser Gly Asn Arg Pro Glu Glu Asp Cys Val Gly Glu Gly Phe Val		
500	505	510
Cys Tyr Ser Leu Cys Ala His Gly His Cys Trp Gly Pro Gly Pro Thr		
515	520	525
Gln Cys Val Asn Cys Ser His Phe Leu Arg Gly Gln Glu Cys Val Glu		
530	535	540
Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg		
545	550	555
560		

-continued

His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val
 565 570 575
 Thr Cys Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr
 580 585 590
 Lys Asp Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro
 595 600 605
 Asp Leu Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala
 610 615 620
 Cys Gln Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp
 625 630 635 640
 Asp Lys Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile
 645 650 655
 Ile Ser Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val
 660 665 670
 Phe Gly Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr
 675 680 685
 Met Arg Arg Asn Glu Asp Leu Gly Pro Ser Ser Pro Met Asp Ser Thr
 690 695 700
 Phe Tyr Arg Ser Leu Leu Glu Asp Glu Asp Met Gly Glu Leu Val Asp
 705 710 715 720
 Ala Glu Glu Tyr Leu Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro
 725 730 735
 Thr Pro Gly Thr Gly Ser Thr Ala His Arg Arg His Arg Ser Ser Ser
 740 745 750
 Ala Arg Asn Gly Gly Asp Leu Thr Leu Gly Met Glu Pro Ser Gly
 755 760 765
 Glu Gly Pro Pro Arg Ser Pro Arg Ala Pro Ser Glu Gly Thr Gly Ser
 770 775 780
 Asp Val Phe Asp Gly Asp Leu Ala Val Gly Val Thr Lys Gly Leu Gln
 785 790 795 800
 Ser Leu Ser Pro Gln Asp Leu Ser Pro Leu Gln Arg Tyr Ser Glu Asp
 805 810 815
 Pro Thr Leu Pro Leu Pro Ser Glu Thr Asp Gly Lys Val Ala Pro Leu
 820 825 830
 Ser Cys Ser Pro Gln Pro Glu Phe Val Asn Gln Ser Asp Val Gln Pro
 835 840 845
 Lys Ser Pro Leu Thr Pro Glu Gly Pro Pro Ser Pro Ala Arg Pro Thr
 850 855 860
 Gly Ala Thr Leu Glu Arg Ala Lys Thr Leu Ser Pro Gly Lys Asn Gly
 865 870 875 880
 Val Val Lys Asp Val Phe Thr Phe Gly Ala Val Glu Asn Pro Glu
 885 890 895
 Phe Leu Ala Pro Arg Glu Gly Thr Ala Ser Pro Pro His Pro Ser Pro
 900 905 910
 Ala Phe Ser Pro Ala Phe Asp Asn Leu Phe Phe Trp Asp Gln Asn Ser
 915 920 925
 Ser Glu Gln Gly Pro Pro Ser Asn Phe Glu Gly Thr Pro Thr Ala
 930 935 940
 Glu Asn Pro Glu Phe Leu Gly Leu Asp Val Pro Val
 945 950 955

-continued

<212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct
 <400> SEQUENCE: 39

atggctagcg agctggccgc	cctgtttaga tggggactgc	tgctggctct gctgctcct	60
ggagccgctt ctacacaggt	ctgcacccggc accgacatga	agctgagact gcccggcagc	120
cccgagacac acctggacat	gctggggcac ctgtaccagg	gctgccaggt ggtccagggg	180
aatcttggaaac tgacctacct	gcccccaac gccagcctga	gtttcctgca ggacatccag	240
gaagtgcagg gctacgtcct	gatcgccac aaccaggctc	gccagggtcc cctgcagcgg	300
ctgagaatcg tgcggggcac	ccagctgttc gaggacaact	acgcctggc cgtgtggac	360
aacggggacc ctctggatag	cgtggccct gctgctgggg	ctacacctgg cggactgcag	420
gaactgcagc tgcggagcct	gaccgagatc ctgaagggcg	gcgtgtgtat cagggggagc	480
cctcagctgt gccaccagga	caccgtgtctg tgggaggacg	tgttccggaa gaacaaccag	540
ctggccctcg tgctgtatgga	caccaacaga agccgggcct	gcacccctcg cgccccatg	600
tgcaaggcca atcaactgt	gggagagagc agccaggact	gcacagaccc gacccggacc	660
atctgcacca ggcctgcgc	cagatgcaag gccccctgc	ctaccgactg ctgccacgaa	720
cagtgegccc ctgggtgcac	cgcccccaag cacagcatt	gcctggctcg cctgcacttc	780
aaccacagcg gcatctgcga	gctgcactgc cctgcctgg	tgacatacaa caccgacacc	840
ttcgagagca tgcccaaccc	cgaggggccgg tacaccttc	gcccggactg tgtgaccgcc	900
tgcccccata actaccttag	cacccgtgt ggcagctgca	ccctgggtgtg cccctgcac	960
aaccaggaaag tgaccgcga	ggacggcacc cagagatgcg	agaagtgcag caaggcttgc	1020
gccagagtgt gctaaggcct	gggcatggaa cacctgagag	aggccagagc catcaccagc	1080
gccaacgtgc aggacttgt	gggctgcaag aagatttcg	gtccctggc cttctgccc	1140
gagagcttcg acggcgatcc	tgcccttcgc accggccctc	tgcagcctga gcagctgcag	1200
gtcttcgaga cactggaga	gatcacccgc tacctgtaca	tcaagcgcctg gcccacagc	1260
ttcccccaacc tgagegtgtt	ccagaacctg agagtgtatcc	ggggcagaat cctgcacaaac	1320
ggcgccata gcctgaccct	gcaggccctg ggaatcagct	ggctggcct gcggagcctg	1380
caggaactgg gatctggcct	ggctctggtg caccggaaacg	cccggtgtg ctctgtgcac	1440
accgtgcctt gggaccagct	gttcagaaac ccccaccagg	ctctgtgca cagcggcaac	1500
cgccccgaag aggattgcgt	gggcgagggc ttctgtgt	actccctgtg cgccacggc	1560
cactgttggg gacctggccc	tacccagtgc gtgaactgca	gccacttcct gcggggccaa	1620
gaatgcgtgg aagagtgcgcg	gggtgtgcag ggactgccc	ggaaatacgt gaacgcccaga	1680
cactgcctgc cttgccaccc	cgagtgcacg ccccagaatg	gcagcgtgac ctgcttcgga	1740
cccgaggccg atcagtgtgt	ggcctgcgc	cactacaagg accccccatt ctgcgtggcc	1800
agatgccccca gcccgcgtgaa	gcccgcacctg agctacatgc	ccatctggaa gttccccgac	1860
gaggaaggcg octgcacagcc	ttgcgcacatc aactgcaccc	acagctgcgt ggacctggac	1920
gacaaggggct gcccgcgcg	gcagagagcc agcccccgt	ccagcatcat cagcgcgtg	1980
gtggaaatcc tgctgggtgt	gggtgtggc gtgtgttgc	gcattctgtat caagcggcgg	2040
cagcagaaga tccggaaagta	caccatgcgg cggAACGAGG	acctggggccc ctctagcccc	2100
atggacagca ctttctaccg	gtccctgtgt	gaagatgagg acatgggcga gctgggtggac	2160

US 9,468,672 B2

319

320

-continued

```

gccgaggaat acctggtgcc tcagcagggc ttcttctgcc ccgaccctac ccctggcacc 2220
ggctctaccg cccacagacg gcacagaacg agcagcgcca gaaacggcg aggcgactg 2280
accctggaa tggAACCTAG CGGCGAGGGA CCTCCCAGAA GCCCTAGAGC CCCTAGCGAG 2340
ggcacccggca gcgacgtgtt cgatggcgat ctggccgtgg gcgtgaccaa gggactgcag 2400
gcctgagcc cccaggacct gtccccctcg cagagataca gcgaggaccc caccctgccc 2460
ctgcccagcg agacagatgg caagggtggcc cccctgagct gcagccctca gcccggatcc 2520
gtgaaccaga gcgacgtgca gcccaagtcc cccctgacac ccgaggggacc tccaagccct 2580
gcccagaccta cggcgccac cctggaaaga gccaagaccc tgagccccgg caagaacggc 2640
gtggtaaaag acgtgttcac cttcggaggc gccgtggaaa accccgagtt cctggccccc 2700
agagaggcga cagccagcccc tccacacccc agcccaagccct tctccccccg cttcgacaac 2760
ctgttcttct gggaccagaa cagcagcgag cagggcccac ccccccagcaa ttgcgaggc 2820
acccccacccg ccgagaatcc tgagttcctg ggctggacg tgcccggtgtg a 2871

```

<210> SEQ ID NO 40

<211> LENGTH: 471

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 40

Met	Asp	Trp	Thr	Trp	Arg	Ile	Leu	Phe	Leu	Val	Ala	Ala	Ala	Thr	Gly
1						5			10					15	

Ala	His	Ser	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys
						20			25				30		

Pro	Gly	Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
						35			40				45		

Thr	Gly	Tyr	Tyr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu
						50			55				60		

Glu	Trp	Met	Gly	Trp	Ile	Asn	Pro	Asp	Ser	Gly	Gly	Thr	Asn	Tyr	Ala
						65			70			75			80

Gln	Lys	Phe	Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Ile	Ser
						85			90			95			

Thr	Ala	Tyr	Met	Glu	Leu	Asn	Arg	Leu	Arg	Ser	Asp	Asp	Thr	Ala	Val
						100			105			110			

Tyr	Tyr	Cys	Ala	Arg	Asp	Gln	Pro	Leu	Gly	Tyr	Cys	Thr	Asn	Gly	Val
						115			120			125			

Cys	Ser	Tyr	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
						130			135			140			

Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser
						145			150			155			160

Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp
						165			170			175			

Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr
						180			185			190			

Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr
						195			200			205			

Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Asn	Phe	Gly	Thr	Gln
						210			215			220			

Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp
						225			230			235			240

-continued

Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala
245 250 255

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
260 265 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
275 280 285

Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
290 295 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
305 310 315 320

Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp
325 330 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
340 345 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg
355 360 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
370 375 380

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385 390 395 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
405 410 415

Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
420 425 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
435 440 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
450 455 460

Leu Ser Leu Ser Pro Gly Lys
465 470

<210> SEQ ID NO 41
<211> LENGTH: 234
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 41

Met Arg Leu Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp Phe Pro
1 5 10 15

Gly Ser Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser
20 25 30

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly
35 40 45

Ile Tyr Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
50 55 60

Asn Leu Leu Ile Tyr Thr Ala Ser Thr Leu Gln Ser Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn
100 105 110

Ile Phe Pro Leu Thr Phe Gly Gly Thr Lys Val Glu Ile Lys Arg
115 120 125

-continued

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> SEQ ID NO 42

<211> LENGTH: 451

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 42

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Arg Gly Ala Thr Leu Tyr Tyr Tyr Tyr Gly Met
100 105 110

Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr
115 120 125

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
130 135 140

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
195 200 205

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
210 215 220

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

-continued

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
290 295 300

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> SEQ ID NO 43
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 43

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Asn Ser Tyr
20 25 30

Leu Asp Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

-continued

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205

Phe Asn Arg Gly Glu Cys
 210

<210> SEQ ID NO 44
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct
<400> SEQUENCE: 44

tgcgtcgaaaaatggtttgcgtttgt cgtt 24

<210> SEQ ID NO 45
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 45

tgcgtcgaaaaatggttt tcggtgcttt t 21

<210> SEQ ID NO 46
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 46

tgcgtcgaaaaatggttt tcggtcgttt t 21

<210> SEQ ID NO 47
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 47

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110

US 9,468,672 B2

329**330**

-continued

Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly
115							120				125				

Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr
130							135				140				

Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn
145						150			155			160			

Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser
165						170			175						

Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly
180						185			190						

Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu
195						200			205						

Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Lys	Glu	Phe
210						215			220						

Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly	Met	Asp	Glu	Leu	Tyr	Lys
225						230			235					

<210> SEQ_ID NO 48

<211> LENGTH: 185

<212> TYPE: PRT

<213> ORGANISM: Orthohepadnavirus hepatitis B virus

<400> SEQUENCE: 48

Met	Asp	Ile	Asp	Pro	Tyr	Lys	Glu	Phe	Gly	Ala	Thr	Val	Glu	Leu	Leu
1						5		10		15					

Ser	Phe	Leu	Pro	Ser	Asp	Phe	Phe	Pro	Ser	Val	Arg	Asp	Leu	Leu	Asp
						20		25		30					

Thr	Ala	Ser	Ala	Leu	Tyr	Arg	Glu	Ala	Leu	Glu	Ser	Pro	Glu	His	Cys
						35		40		45					

Ser	Pro	His	His	Thr	Ala	Leu	Arg	Gln	Ala	Ile	Leu	Cys	Trp	Gly	Glu
						50		55		60					

Leu	Met	Thr	Leu	Ala	Thr	Trp	Val	Gly	Asn	Asn	Leu	Glu	Asp	Pro	Ala
65						70		75		80					

Ser	Arg	Asp	Leu	Val	Val	Asn	Tyr	Val	Asn	Thr	Asn	Met	Gly	Leu	Lys
						85		90		95					

Ile	Arg	Gln	Leu	Leu	Trp	Phe	His	Ile	Ser	Cys	Leu	Thr	Phe	Gly	Arg
						100		105		110					

Glu	Thr	Val	Leu	Glu	Tyr	Leu	Val	Ser	Phe	Gly	Val	Trp	Ile	Arg	Thr
						115		120		125					

Pro	Pro	Ala	Tyr	Arg	Pro	Pro	Asn	Ala	Pro	Ile	Leu	Ser	Thr	Leu	Pro
130						135		140							

Glu	Thr	Thr	Val	Val	Arg	Arg	Arg	Gly	Arg	Gly	Arg	Ser	Pro	Arg	Arg
145						150		155		160					

Arg	Thr	Pro	Ser	Pro	Arg	Arg	Arg	Ser	Gln	Ser	Pro	Arg	Arg	Arg	
						165		170		175					

Arg	Ser	Gln	Ser	Arg	Glu	Ser	Gln	Cys							
						180		185							

<210> SEQ_ID NO 49

<211> LENGTH: 226

<212> TYPE: PRT

<213> ORGANISM: Orthohepadnavirus hepatitis B virus

<400> SEQUENCE: 49

Met	Glu	Asn	Ile	Thr	Ser	Gly	Phe	Leu	Gly	Pro	Leu	Leu	Val	Leu	Gln
1						5		10		15					

US 9,468,672 B2

331**332**

-continued

Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu
 20 25 30

Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ser Pro Val Cys
 35 40 45

Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser
 50 55 60

Cys Pro Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe
 65 70 75 80

Ile Ile Phe Leu Ile Leu Leu Cys Leu Ile Phe Leu Leu Val
 85 90 95

Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly
 100 105 110

Ser Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala
 115 120 125

Gln Gly Asn Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp
 130 135 140

Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys
 145 150 155 160

Tyr Leu Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu
 165 170 175

Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu
 180 185 190

Ser Ala Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile
 195 200 205

Val Ser Pro Phe Ile Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val
 210 215 220

Tyr Ile
 225

<210> SEQ_ID NO 50
 <211> LENGTH: 737
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 50

Met Ala Ser Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp
 1 5 10 15

Val Pro Gly Ser Thr Gly Asp Ala Ala His His His His His Lys
 20 25 30

Ser Ser Ser Glu Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys Ala
 35 40 45

Phe Leu Asp Glu Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu His Asn
 50 55 60

Phe Thr Gln Ile Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln Leu
 65 70 75 80

Ala Lys Gln Ile Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser Val
 85 90 95

Glu Leu Thr His Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr His
 100 105 110

Pro Asn Tyr Ile Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn
 115 120 125

Thr Ser Leu Phe Glu Pro Pro Pro Ala Gly Tyr Glu Asn Val Ser Asp
 130 135 140

-continued

Ile Val Pro Pro Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu Gly
145 150 155 160

Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu
165 170 175

Glu Arg Asp Met Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala Arg
180 185 190

Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu Ala
195 200 205

Gly Ala Thr Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala
210 215 220

Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly
225 230 235 240

Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu
245 250 255

Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala
260 265 270

Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr Tyr
275 280 285

Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Ser Pro Asp
290 295 300

Ser Ser Trp Arg Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro Gly
305 310 315 320

Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met His Ile His Ser
325 330 335

Thr Ser Glu Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly
340 345 350

Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp Ser
355 360 365

Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val His
370 375 380

Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro
385 390 395 400

Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu
405 410 415

Leu Gly Ser Thr Glu Trp Ala Glu Asn Ser Arg Leu Leu Gln Glu
420 425 430

Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr
435 440 445

Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val Tyr Asn
450 455 460

Leu Thr Lys Glu Leu Glu Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser
465 470 475 480

Leu Tyr Glu Ser Trp Thr Lys Ser Pro Ser Pro Glu Phe Ser Gly Met
485 490 495

Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val Phe Phe
500 505 510

Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp
515 520 525

Glu Thr Asn Lys Phe Ser Ser Tyr Pro Leu Tyr His Ser Val Tyr Glu
530 535 540

Thr Tyr Glu Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys Tyr His
545 550 555 560

Leu Thr Val Ala Gln Val Arg Gly Met Val Phe Glu Leu Ala Asn

-continued

565	570	575
-----	-----	-----

Ser Val Val Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg
580 585 590

Lys Tyr Ala Asp Lys Ile Tyr Asn Ile Ser Met Lys His Pro Gln Glu
595 600 605

Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys
610 615 620

Asn Phe Thr Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Arg Asp Phe
625 630 635 640

Asp Lys Ser Asn Pro Ile Leu Leu Arg Met Met Asn Asp Gln Leu Met
645 650 655

Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro
660 665 670

Phe Tyr Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala
675 680 685

Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser
690 695 700

Lys Val Asp Pro Ser Gln Ala Trp Gly Glu Val Lys Arg Gln Ile Ser
705 710 715 720

Ile Ala Thr Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val
725 730 735

Ala

<210> SEQ ID NO 51

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 51

Thr Tyr Val Pro Ala Asn Ala Ser Leu
1 5

<210> SEQ ID NO 52

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 52

Asp Met Val Leu Trp Lys Asp Val Phe Arg Lys Asn Asn Gln Leu
1 5 10 15

<210> SEQ ID NO 53

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 53

Ser Tyr Val Asn Thr Asn Met Gly Leu
1 5

<210> SEQ ID NO 54

<211> LENGTH: 957

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 54

Met Ala Ser Glu Leu Ala Ala Trp Cys Arg Trp Gly Phe Leu Leu Ala			
1	5	10	15
Leu Leu Pro Pro Gly Ile Ala Gly Thr Gln Val Cys Thr Gly Thr Asp			
20	25	30	
Met Lys Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu			
35	40	45	
Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu			
50	55	60	
Thr Tyr Val Pro Ala Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln			
65	70	75	80
Glu Val Gln Gly Tyr Met Leu Ile Ala His Asn Gln Val Lys Arg Val			
85	90	95	
Pro Leu Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp			
100	105	110	
Lys Tyr Ala Leu Ala Val Leu Asp Asn Arg Asp Pro Gln Asp Asn Val			
115	120	125	
Ala Ala Ser Thr Pro Gly Arg Thr Pro Glu Gly Leu Arg Glu Leu Gln			
130	135	140	
Leu Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Arg Gly			
145	150	155	160
Asn Pro Gln Leu Cys Tyr Gln Asp Met Val Leu Trp Lys Asp Val Phe			
165	170	175	
Arg Lys Asn Asn Gln Leu Ala Pro Val Asp Ile Asp Thr Asn Arg Ser			
180	185	190	
Arg Ala Cys Pro Pro Cys Ala Pro Ala Cys Lys Asp Asn His Cys Trp			
195	200	205	
Gly Glu Ser Pro Glu Asp Cys Gln Ile Leu Thr Gly Thr Ile Cys Thr			
210	215	220	
Ser Gly Cys Ala Arg Cys Lys Gly Arg Leu Pro Thr Asp Cys Cys His			
225	230	235	240
Glu Gln Cys Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu			
245	250	255	
Ala Cys Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro			
260	265	270	
Ala Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met His Asn Pro			
275	280	285	
Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Thr Cys Pro Tyr			
290	295	300	
Asn Tyr Leu Ser Thr Glu Val Gly Ser Cys Thr Leu Val Cys Pro Pro			
305	310	315	320
Asn Asn Gln Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys			
325	330	335	
Cys Ser Lys Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His			
340	345	350	
Leu Arg Gly Ala Arg Ala Ile Thr Ser Asp Asn Val Gln Glu Phe Asp			
355	360	365	
Gly Cys Lys Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe			
370	375	380	
Asp Gly Asp Pro Ser Ser Gly Ile Ala Pro Leu Arg Pro Glu Gln Leu			
385	390	395	400

-continued

Gln Val Phe Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser
405 410 415

Ala Trp Pro Asp Ser Leu Arg Asp Leu Ser Val Phe Gln Asn Leu Arg
420 425 430

Ile Ile Arg Gly Arg Ile Leu His Asp Gly Ala Tyr Ser Leu Thr Leu
435 440 445

Gln Gly Leu Gly Ile His Ser Leu Gly Leu Arg Ser Leu Arg Glu Leu
450 455 460

Gly Ser Gly Leu Ala Leu Ile His Arg Asn Ala His Leu Cys Phe Val
465 470 475 480

His Thr Val Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu
485 490 495

Leu His Ser Gly Asn Arg Pro Glu Glu Asp Cys Gly Leu Glu Gly Leu
500 505 510

Val Cys Asn Ser Leu Cys Ala His Gly His Cys Trp Gly Pro Gly Pro
515 520 525

Thr Gln Cys Val Asn Cys Ser His Phe Leu Arg Gly Gln Glu Cys Val
530 535 540

Glu Glu Cys Arg Val Trp Lys Gly Leu Pro Arg Glu Tyr Val Ser Asp
545 550 555 560

Lys Arg Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Ser Ser
565 570 575

Glu Thr Cys Phe Gly Ser Glu Ala Asp Gln Cys Ala Ala Cys Ala His
580 585 590

Tyr Lys Asp Ser Ser Ser Cys Val Ala Arg Cys Pro Ser Gly Val Lys
595 600 605

Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys Tyr Pro Asp Glu Glu Gly
610 615 620

Ile Cys Gln Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu
625 630 635 640

Asp Glu Arg Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Val Thr Phe
645 650 655

Ile Ile Ala Thr Val Val Gly Val Leu Leu Phe Leu Ile Leu Val Val
660 665 670

Val Val Gly Ile Leu Ile Lys Arg Arg Arg Gln Lys Ile Arg Lys Tyr
675 680 685

Thr Met Arg Arg Asn Glu Asp Leu Gly Pro Ser Ser Pro Met Asp Ser
690 695 700

Thr Phe Tyr Arg Ser Leu Leu Glu Asp Asp Met Gly Asp Leu Val
705 710 715 720

Asp Ala Glu Glu Tyr Leu Val Pro Gln Gln Gly Phe Phe Ser Pro Asp
725 730 735

Pro Thr Pro Gly Thr Gly Ser Thr Ala His Arg Arg His Arg Ser Ser
740 745 750

Ser Thr Arg Ser Gly Gly Glu Leu Thr Leu Gly Leu Glu Pro Ser
755 760 765

Glu Glu Gly Pro Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly
770 775 780

Ser Asp Val Phe Asp Gly Asp Leu Ala Met Gly Val Thr Lys Gly Leu
785 790 795 800

Gln Ser Leu Ser Pro His Asp Leu Ser Pro Leu Gln Arg Tyr Ser Glu
805 810 815

Asp Pro Thr Leu Pro Leu Pro Pro Glu Thr Asp Gly Tyr Val Ala Pro

-continued

820	825	830
Leu Ala Cys Ser Pro Gln Pro Glu Phe Val Asn Gln Ser Glu Val Gln		
835	840	845
Pro Gln Pro Pro Leu Thr Pro Glu Gly Pro Leu Pro Pro Val Arg Pro		
850	855	860
Ala Gly Ala Thr Leu Glu Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn		
865	870	875
Gly Val Val Lys Asp Val Phe Ala Phe Gly Gly Ala Val Glu Asn Pro		
885	890	895
Glu Phe Leu Val Pro Arg Glu Gly Thr Ala Ser Pro Pro His Pro Ser		
900	905	910
Pro Ala Phe Ser Pro Ala Phe Asp Asn Leu Phe Phe Trp Asp Gln Asn		
915	920	925
Ser Ser Glu Gln Gly Pro Pro Pro Ser Asn Phe Glu Gly Thr Pro Thr		
930	935	940
Ala Glu Asn Pro Glu Phe Leu Gly Leu Asp Val Pro Val		
945	950	955

```
<210> SEQ ID NO 55
<211> LENGTH: 739
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct
```

<400> SEQUENCE: 55
 Met Ala Ser Ala Arg Arg Pro Arg Trp Leu Cys Ala Gly Ala Leu Val
 1 5 10 15
 Leu Ala Gly Gly Phe Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe Ile
 20 25 30
 Lys Ser Ser Ser Glu Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys
 35 40 45
 Ala Phe Leu Asp Glu Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu His
 50 55 60
 Asn Phe Thr Gln Ile Pro His Leu Ala Gly Thr Glu Gln Asn Phe Gln
 65 70 75 80
 Leu Ala Lys Gln Ile Gln Ser Gln Trp Lys Glu Phe Gly Leu Asp Ser
 85 90 95
 Val Glu Leu Thr His Tyr Asp Val Leu Leu Ser Tyr Pro Asn Lys Thr
 100 105 110
 His Pro Asn Tyr Ile Ser Ile Ile Asn Glu Asp Gly Asn Glu Ile Phe
 115 120 125
 Asn Thr Ser Leu Phe Glu Pro Pro Pro Ala Gly Tyr Glu Asn Val Ser
 130 135 140
 Asp Ile Val Pro Pro Phe Ser Ala Phe Ser Pro Gln Gly Met Pro Glu
 145 150 155 160
 Gly Asp Leu Val Tyr Val Asn Tyr Ala Arg Thr Glu Asp Phe Phe Lys
 165 170 175
 Leu Glu Arg Asp Met Lys Ile Asn Cys Ser Gly Lys Ile Val Ile Ala
 180 185 190
 Arg Tyr Gly Lys Val Phe Arg Gly Asn Lys Val Lys Asn Ala Gln Leu
 195 200 205
 Ala Gly Ala Thr Gly Val Ile Leu Tyr Ser Asp Pro Ala Asp Tyr Phe
 210 215 220
 Ala Pro Gly Val Lys Ser Tyr Pro Asp Gly Trp Asn Leu Pro Gly Gly

-continued

225	230	235	240
<hr/>			
Gly Val Gln Arg Gly Asn Ile Leu Asn Leu Asn Gly Ala Gly Asp Pro			
245	250	255	
Leu Thr Pro Gly Tyr Pro Ala Asn Glu Tyr Ala Tyr Arg Arg Gly Ile			
260	265	270	
Ala Glu Ala Val Gly Leu Pro Ser Ile Pro Val His Pro Ile Gly Tyr			
275	280	285	
Tyr Asp Ala Gln Lys Leu Leu Glu Lys Met Gly Gly Ser Ala Ser Pro			
290	295	300	
Asp Ser Ser Trp Arg Gly Ser Leu Lys Val Pro Tyr Asn Val Gly Pro			
305	310	315	320
Gly Phe Thr Gly Asn Phe Ser Thr Gln Lys Val Lys Met His Ile His			
325	330	335	
Ser Thr Ser Glu Val Thr Arg Ile Tyr Asn Val Ile Gly Thr Leu Arg			
340	345	350	
Gly Ala Val Glu Pro Asp Arg Tyr Val Ile Leu Gly Gly His Arg Asp			
355	360	365	
Ser Trp Val Phe Gly Gly Ile Asp Pro Gln Ser Gly Ala Ala Val Val			
370	375	380	
His Glu Ile Val Arg Ser Phe Gly Thr Leu Lys Lys Glu Gly Trp Arg			
385	390	395	400
Pro Arg Arg Thr Ile Leu Phe Ala Ser Trp Asp Ala Glu Glu Phe Gly			
405	410	415	
Leu Leu Gly Ser Thr Glu Trp Ala Glu Glu Asn Ser Arg Leu Leu Gln			
420	425	430	
Glu Arg Gly Val Ala Tyr Ile Asn Ala Asp Ser Ser Ile Glu Gly Asn			
435	440	445	
Tyr Thr Leu Arg Val Asp Cys Thr Pro Leu Met Tyr Ser Leu Val Tyr			
450	455	460	
Asn Leu Thr Lys Glu Leu Glu Ser Pro Asp Glu Gly Phe Glu Gly Lys			
465	470	475	480
Ser Leu Tyr Glu Ser Trp Thr Lys Ser Pro Ser Pro Glu Phe Ser			
485	490	495	
Gly Met Pro Arg Ile Ser Lys Leu Gly Ser Gly Asn Asp Phe Glu Val			
500	505	510	
Phe Phe Gln Arg Leu Gly Ile Ala Ser Gly Arg Ala Arg Tyr Thr Lys			
515	520	525	
Asn Trp Glu Thr Asn Lys Phe Ser Ser Tyr Pro Leu Tyr His Ser Val			
530	535	540	
Tyr Glu Thr Tyr Glu Leu Val Glu Lys Phe Tyr Asp Pro Met Phe Lys			
545	550	555	560
Tyr His Leu Thr Val Ala Gln Val Arg Gly Gly Met Val Phe Glu Leu			
565	570	575	
Ala Asn Ser Val Val Leu Pro Phe Asp Cys Arg Asp Tyr Ala Val Val			
580	585	590	
Leu Arg Lys Tyr Ala Asp Lys Ile Tyr Asn Ile Ser Met Lys His Pro			
595	600	605	
Gln Glu Met Lys Thr Tyr Ser Val Ser Phe Asp Ser Leu Phe Ser Ala			
610	615	620	
Val Lys Asn Phe Thr Glu Ile Ala Ser Lys Phe Ser Glu Arg Leu Arg			
625	630	635	640
Asp Phe Asp Lys Ser Asn Pro Ile Leu Leu Arg Met Met Asn Asp Gln			
645	650	655	

-continued

Leu Met Phe Leu Glu Arg Ala Phe Ile Asp Pro Leu Gly Leu Pro Asp
660 665 670

Arg Pro Phe Tyr Arg His Val Ile Tyr Ala Pro Ser Ser His Asn Lys
675 680 685

Tyr Ala Gly Glu Ser Phe Pro Gly Ile Tyr Asp Ala Leu Phe Asp Ile
690 695 700

Glu Ser Lys Val Asp Pro Ser Gln Ala Trp Gly Glu Val Lys Arg Gln
705 710 715 720

Ile Ser Ile Ala Thr Phe Thr Val Gln Ala Ala Ala Glu Thr Leu Ser
725 730 735

Glu Val Ala

<210> SEQ_ID NO 56

<211> LENGTH: 2217

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 56

atggcttagcg	ctagaaggcc	cagatggctg	tgcgctggcg	ccctgggtct	ggctggcgga	60
ttcttcttc	ttggcttc	tttcggctgg	ttcatcaagt	cctccagcga	ggccaccaac	120
atcaccccca	agcacaacat	gaaggccctt	ctggacgagc	tgaaggccga	aatatcaag	180
aagttctgc	acaacttcac	ccagatcccc	cacctggccc	gcacccgagca	gaacttcag	240
ctggccaagc	agatccagtc	ccagtggaaa	gagttcgcc	tggactccgt	ggaactgacc	300
cactacgacg	tgctgctgtc	ctaccccaac	aagacccacc	ccaactacat	ctccatcatc	360
aacgaggacg	gcaacgaaat	cttcaacacc	tccctgttc	agccccacc	agccggctac	420
gagaacgtgt	ccgacatcgt	gcccccattc	tccgcattca	gtccacaagg	catgcccag	480
ggcgacctgg	tgtacgtgaa	ctacgcccagg	accgaggact	tcttcaagct	ggaaaggagc	540
atgaagatca	actgctccgg	caagatcgtg	atcgccagat	acggcaaggt	gttcaggggc	600
aacaaagtga	agaacgctca	gctggctggg	gccacccggc	tgatcctgta	ctctgacccc	660
gccgactact	tcgccccagg	cgtgaagtcc	taccccgacg	gttggaaacct	gccaggtggc	720
ggagtgcaga	ggggcaacat	cctgaacctg	aacggcgctg	gctggccct	gaccccgagga	780
taccccgcca	acgagtaacgc	ctacagaaga	ggaatcgccg	aggccgtggg	cctgcctct	840
atcccagtgc	accccatcg	ctactacgac	gcccagaaac	tgctggaaaa	gatggccgc	900
tccgcctccc	ccgactcctc	ttggagaggc	tccctgaagg	tgccttacaa	cgtggccca	960
ggcttcacccg	gcaacttctc	cacccagaaa	gtgaagatgc	acatccactc	caccccgaa	1020
gtgaccagga	tctacaacgt	gatggccacc	ctgagaggcg	ccgttggaaacc	cgacagatac	1080
gtgatcctgg	ggggccacag	ggacagctgg	gtgttccggc	gcatcgaccc	acagtctggc	1140
cccgctgtgg	tgcacagat	cgtcggtcc	ttcggaaacc	tgaagaaaga	gggatggcgc	1200
cccagaagga	caatcctgtt	cgcctctgg	gacgcccagg	aattcggcct	gctggatcc	1260
accgagtggg	ccgaggaaaa	ctccaggctg	ctgcaggaaa	ggggcgctcg	ctacatcaac	1320
gccgactct	ccatcgaggg	caactacacc	ctgagggtgg	actgcacccc	cctgtatgtac	1380
tccctggtgt	acaacctgac	caaagagctg	gaatcccccc	acgagggttt	cgaggccaag	1440
tccctgtacg	agtccctggac	caagaagtcc	ccatcccccc	agttctccgg	catgcccagg	1500
atctccaagc	tggttccgg	caacgacttc	gagggtttct	tccagaggct	ggaaatcgcc	1560

-continued

tccggcaggg ccagatacac caagaactgg gagacaaaca agtttccttc ctacccctg	1620
taccactcg tgtacgaaac ctacgagctg gtggaaaagt tctacgaccc catgttcaag	1680
taccacctga ccgtggccca ggtccgcgga ggcatggtgt tcgagctggc caactccgtg	1740
gtgctgcct tcgactgcag agactatgt gtggtgctga ggaagtacgc cgacaaaatc	1800
tacaacatct ccatgaagca cccccaggaa atgaagacct actccgtgtc cttcgactcc	1860
ctgttctccg ccgtgaagaa ttccaccgag atcgcctcca agttctccga gaggtggagg	1920
gacttcgaca agtccaaccc catcctgtg aggatgtga acgaccagct gatgttctg	1980
gaaaggccct tcatcgaccc cctggccctg ccagacaggc ccttctacag gcacgtgatc	2040
tacgccccat cctcccacaa caaatacgcc ggcgagtccct tcccccggcat ctacgatgcc	2100
ctgttcgaca tcgagtccaa ggtggacccc tcccaggett ggggcaagt gaagaggcag	2160
atcagtatcg ccacattcac agtgcaggcc gctgccgaaa ccctgtccga ggtggcc	2217

<210> SEQ ID NO 57

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Foot-and-mouth disease virus

<400> SEQUENCE: 57

Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val Glu Ser			
1	5	10	15

Asn Pro Gly Pro	
20	

<210> SEQ ID NO 58

<211> LENGTH: 18

<212> TYPE: PRT

<213> ORGANISM: Thosea Asigna Virus

<400> SEQUENCE: 58

Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro			
1	5	10	15

Gly Pro

<210> SEQ ID NO 59

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Encephalomyocarditis virus

<400> SEQUENCE: 59

His Tyr Ala Gly Tyr Phe Ala Asp Leu Leu Ile His Asp Ile Glu Thr			
1	5	10	15

Asn Pro Gly Pro	
20	

<210> SEQ ID NO 60

<211> LENGTH: 20

<212> TYPE: PRT

<213> ORGANISM: Equine Rhinitis A Virus

<400> SEQUENCE: 60

Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser			
1	5	10	15

Asn Pro Gly Pro	
20	

-continued

<210> SEQ ID NO 61
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Porcine Teschovirus

<400> SEQUENCE: 61

```
Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn
1           5           10          15

Pro Gly Pro
```

<210> SEQ ID NO 62
<211> LENGTH: 568
<212> TYPE: RNA
<213> ORGANISM: Encephalomyocarditis virus

<400> SEQUENCE: 62

```
uaacguuacu ggccgaagcc gcuuggaaua aggccggugu gcgguuugucu auauguauuu       60
uucccacaua uugccgucuu uuggcaaugu gaggggcccg aaaccuggcc cugucuuucuu      120
gacgagcauu ccuagggguc uuuccccucu cgccaaaggga augcaagguc uguugaaugu      180
cgugaaggaa gcaguuccuc uggaagcuuc uugaagacaa acaacgucug uagcgacccu      240
uugcaggcag cggAACCCCCC caccuggcga caggugccuc ugcccggccaaa agccacgugu      300
auaagauaca ccugcaaagg cggcacaaacc ccagugccac guugugaguu ggauaguugu      360
ggaaagaguc aaauggcucu ccucaagcgu auucaacaag gggcugaagg augcccagaa      420
gguaacccau uguauugggau cugaucuggg gcccugugc acaugcuaaua cauguguuuu      480
gucgaggua aaaaacgucu aggccccccg aaccacgggg acgugguuuu ccuuugaaaa      540
acacgaugau aauauggcca caaccaug                                         568
```

The invention claimed is:

1. A method of treating prostate cancer in a human, comprising administering to the human an effective amount of a composition 1 comprising a multi-antigen construct, 40 wherein the multi-antigen construct comprises:
 - (a) at least one nucleotide sequence encoding an immunogenic PSA polypeptide;
 - (b) at least one nucleotide sequence encoding an immunogenic PSCA polypeptide; and
 - (c) at least one nucleotide sequence encoding an immunogenic PSMA polypeptide, wherein the immunogenic PSA polypeptide comprises amino acids 4-240 of SEQ ID NO:17, wherein the immunogenic PSCA polypeptide comprises the amino acid sequence of SEQ ID NO:21, and wherein the immunogenic PSMA polypeptide has at least 90% identity with amino acids 15-750 of the human PSMA of SEQ ID NO:1 and comprises the amino acids of at least 10 conserved T cell epitopes of the human PSMA at corresponding positions.
 2. The method according to claim 1, wherein the immunogenic PSMA polypeptide is selected from the group consisting of:
 - 1) a polypeptide comprising amino acids 15-750 of SEQ ID NO: 1;
 - 2) a polypeptide comprising the amino acid sequence of SEQ ID NO:3;
 - 3) a polypeptide comprising the amino acid sequence of SEQ ID NO:5;
 - 4) a polypeptide comprising the amino acid sequence of SEQ ID NO:7;
 - 5) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:9;
 - 6) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:3;
 - 7) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:5;
 - 8) a polypeptide comprising the amino acids 4-739 of SEQ ID NO:7; and
 - 9) polypeptide comprising the amino acid sequence of SEQ ID NO: 9.
3. The method according to claim 2, wherein the nucleotide sequence encoding the immunogenic PSA polypeptide is set forth in SEQ ID NO:18.
4. The method according to claim 3, wherein the nucleotide sequence encoding the immunogenic PSMA polypeptide is selected from the group consisting of:
 - 1) the nucleotide sequence of SEQ ID NO:2;
 - 2) the nucleotide sequence of SEQ ID NO:4;
 - 3) the nucleotide sequence of SEQ ID NO:6;
 - 4) the nucleotide sequence of SEQ ID NO:8;
 - 5) the nucleotide sequence of SEQ ID NO:10;
 - 6) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:4;
 - 7) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:6;
 - 8) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:8; and
 - 9) a nucleotide sequence comprising nucleotides 10-2217 of SEQ ID NO:10.

5. The method according to claim 1, wherein the multi-antigen construct is incorporated into a vector.

351

6. The method according to claim 5, wherein the multi-antigen construct further comprises:

- (a) a nucleotide sequence encoding a T2A peptide sequence; and
- (b) a nucleotide sequence encoding a F2A peptide sequence.

7. The method according to 6, wherein the order of the nucleotide sequences on the multi-antigen construct is shown in formula (I):

PSA-T2A-PSCA-F2A-PSMA (I)

wherein in formula (I):

PSA is the nucleotide sequence encoding the immunogenic PSA polypeptide;

PSCA is the nucleotide sequence encoding the immunogenic PSCA polypeptide;

PSMA is the nucleotide sequence encoding the immunogenic PSMA polypeptide;

T2A is the nucleotide sequence encoding the T2A peptide sequence; and

F2A is the nucleotide sequence encoding the F2A peptide sequence.

8. The method according to 7, wherein the immunogenic PSMA polypeptide comprises the amino acid sequence of SEQ ID NO: 9.

9. The method according to claim 7, wherein the nucleotide sequence encoding the immunogenic PSMA polypeptide is set forth in SEQ ID NO:10.

10. A method of treating prostate cancer in a human, comprising administering to the human an effective amount

352

of a composition comprising multi-antigen construct, wherein the multi-antigen construct comprises the nucleotide sequence of SEQ ID NO:35 or a degenerate variant of the nucleotide sequence of SEQ ID NO:35.

5 11. The method according to claim 10, wherein the multi-antigen construct comprises a degenerate variant of the nucleotide sequence of SEQ ID NO:35.

12. The method according to claim 10, further comprising administering to the human an effective amount of an 10 immune modulators.

13. The method according to claim 12, wherein the immune modulator is an immune-effector-cell enhancer.

14. The method according to claim 13, wherein the immune-effector-cell enhancer is selected from the group 15 consisting of TNFR agonists, CTLA-4 antagonists, TLR agonists, programmed cell death protein 1 antagonists, programmed cell death protein 1 ligand antagonists.

15. The method according to claim 14, wherein the immune-effector-cell enhancer is a CTLA-4 antagonist.

20 16. The method according to claim 15, wherein the CTLA-4 antagonist is an anti-CTLA-4 antibody.

17. The method according to claim 14, wherein the immune-effector-cell enhancer is a TLR agonist.

25 18. The method according to claim 17, wherein the TLR agonist is a CpG oligonucleotide.

19. The method according to claim 12, wherein the immune modulator is an immune-suppressive-cell inhibitor.

20 20. The method according to claim 19, wherein the immune-suppressive-cell inhibitor is sunitinib malate.

* * * * *